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The Phylogeny of the Prasinophyceae and Pleurastrophyceae (Chlorophyta) Inferred From Ribosomal RNA Genes and Morphology.

Thomas Sinclair Kantz
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The phylogeny of the Prasinophyceae and Pleurastrophyceae (Chlorophyta) inferred from ribosomal RNA genes and morphology

Kantz, Thomas Sinclair, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1993

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THE PHYLOGENY OF THE PRASINOPHYCEAE AND PLEURASTROPHYCEAE (CHLOROPHYTA) INFERRED FROM RIBOSOMAL RNA GENES AND MORPHOLOGY

A Dissertation

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Louisiana State University and
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in

The Department of Botany

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ABSTRACT

The phylogeny of the problematic green algal classes Prasinophyceae and Pleurastrophyceae (Chlorophyta) sensu Mattox and Stewart (1984) was inferred from ultrastructural and biochemical data, and from ribosomal RNA gene sequence data.

Analysis of a data set of ultrastructural and biochemical characters (with a predominance of flagellar and cell covering features) revealed a monophyletic Pleurastrophyceae and a monophyletic Prasinophyceae. Rerooting experiments indicated that the ancestral flagellate was likely Pedinomonas-like or Mamiellales-like.

The cladograms generated from the ribosomal RNA gene sequence data show that the Pleurastrophyceae is not monophyletic; however, pleurastrophycean taxa are more closely related to the Chlorophyceae than to any other class. The Ulvophyceae is the sister group to the Chlorophyceae plus Pleurastrophyceae clade. The Charophyceae and land plants represent a basal divergence relative to the Chlorophyceae plus

Pleurastrophyceae plus Ulvophyceae clade. The Prasinophyceae is not a monophyletic group -- some prasinophycean taxa are more closely allied to the other classes of green algae than with each other. User-defined topologies indicate that the ancestral flagellate was likely Mamiellales-like or Pyramimonadales-like. Analysis of a combined data set favored a Mamiellales-like ancestor. Despite the antiquity of the green algal lineages, randomization tests of the sequence data show a high level of phylogenetic signal and a low level of randomness in the data.

A preliminary study of subgeneric relationships of the asexual green flagellate Tetraselmis was performed using sequences from polymerase chain reaction amplified internal transcribed spacer (ITS) regions 1 and 2 and the 5.8S rDNA gene. The 5.8S gene sequence was of marginal use in resolving relationships among the subgenera. The ITS regions were variable enough to resolve relationships among the three subgenera.

INTRODUCTION

The green algae are an ancient and diverse lineage that provide evolutionary biologists with many taxonomic and phylogenetic challenges. A major source of phylogenetic information has been morphological and biochemical features, including type of flagellation, life cycle, storage products, wall components, and photosynthetic pigments. Appreciation for the diversity of the green algae deepened with the advent of transmission electron microscope (TEM) studies, which revealed many new phylogenetically informative characters. Many of these ultrastructural features (e.g., features of mitosis, cytokinesis, and the flagellar apparatus) are thought to be evolutionarily conservative and form the basis for several classification schemes for the green algae (Mattox and Stewart 1984, O'Kelly and Floyd 1984, Melkonian 1984, van den Hoek et al. 1989).

The differing classifications have led to conflicting hypotheses of green algal evolution. The

conflicts surrounding the two problematic classes Prasinophyceae and Pleurastrophyceae are the focus of this dissertation. Fundamental disagreement exists regarding whether the Prasinophyceae is monophyletic or comprises several lineages, what the major lineages within the assemblage are, and which lineages are ancestral to the rest of the green algae. Likewise, the monophyly of the Pleurastrophyceae, the major lineages within the class, and its phylogenetic relationship to other green algal classes is a matter of contention.

The conflicting hypotheses of evolution based on morphological, biochemical, and ultrastructural features can be tested with an explicit cladistic analysis of an independent suite of characters. Because ribosomal RNA gene sequences represent an independent data set, are found in all organisms, and are evolutionarily conserved, the use of rRNA gene sequences in a phylogenetic analysis of the green algae seems particularly appropriate.

A major problem in phylogenetic studies of the green algae is the choice of an appropriate outgroup. It is likely that all flagellate algal groups evolved

from zooflagellates (Stewart and Mattox 1980, Round 1984, Mattox and Stewart 1984); however, the zooflagellate group from which the green algae evolved is unclear. Mattox and Stewart (1984) reviewed cytological features that they considered primitive for the green algae with the goal of finding a likely zooflagellate ancestral group that shared these features. The putatively primitive cytological features included a covering of scales instead of a wall or theca, an interzonal mitotic spindle that persists during cytokinesis, a depression or pit at the point of flagellar insertion, the "H-piece" of the flagellar transition region, an asymmetric flagellar root system, and a microtubular multilayered structure (MLS) associated with the flagellar root. They concluded that there is no known zooflagellate group with these features that would closely link it to the Chlorophyta.

Sleigh (1988) compared the flagellar root patterns in 25 protists, including ciliates, zooflagellates, and several algal groups. Although he did not discuss possible evolutionary relationships among the taxa, he did note structural

similarities of the flagellar apparatus of the prasinophyte Nephroselmis with that of the chrysophytes and bodonids. However, it is not known whether the similarities are synapomorphies or symplesiomorphies.

Lee and Kugrens (1991) described a colorless flagellate Katablepharis ovalis with features similar to those in the Cryptophyceae. Interestingly, it also has a scaly covering and ejectosomes similar in some ways to those found in Pyramimonas and other prasinophytes. The presence of ejectosomes in Pyramimonas and the Cryptophyceae also led Taylor (1976, 1978) to suggest a close evolutionary relationship; however, one should not place too much emphasis on the presence of ejectosomes, because a close comparison of the ejectosomes in the two groups showed fundamental differences in their structures, and they may not be evolutionarily homologous (Morrall and Greenwood 1980).

Several molecular studies have suggested possible sister groups to the green algae. Bhattacharya and Dreuhl (1988) performed a phenetic analysis of small subunit (SSU) ribosomal RNA

sequences from some chlorophyll a + b and chlorophyll a + c possessing organisms. In the resulting phenogram the brown alga Costaria costata, the oomycete fungus Achlya bisexualis, and the chrysophyte Ochromonas danica formed a clade that was the sister group to the green algae and land plants.

In a phenetic analysis of SSU rRNA sequences focusing on chromophyte algal relationships, Ariztia et al. (1991) showed a phenogram with the amoeboid protozoan Acanthamoeba castellanii as the sister group to the green algae and land plants. However, the branch supporting this relationship was very short and statistically uncertain. The sister clade to the Acanthamoeba and green plant clade was a clade with animals and the ascomycete fungus Saccharomyces cerevisiae.

Bhattacharya et al. (1991) performed a cladistic analysis of actin gene sequences from a wide range of taxa, including green algae and land plants. They presented an unrooted cladogram, which makes determination of sister group relationships difficult because the clade of green algae and land plants emerge from a trichotomy. The other two clades of

the trichotomy are possible sister groups to the green plants; which clade is the sister group depends on the position of the root. One of the possible sister group clades includes animals, Saccharomyces cereviseae, Acanthamoeba castellanii, and other amoeboid protozoans. The other possible sister group to the green algae includes Costaria costata, Achlya bisexualis, the zooflagellate Trypanosoma brucei, and other flagellated and ciliated protozoans.

Clearly, the identity of the sister group to the green algae and land plants requires further study. Neither the ultrastructural nor the molecular data indicate a clear-cut relationship of the green algae with any other single lineage. For the following studies of green algal evolution, several possible sister groups were chosen to root the cladograms. These outgroups were Saccharomyces cereviseae of the Ascomycota, Costaria costata of the Phaeophyta, and the diatom Phaeodactylum of the Chrysophyta.

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CHAPTER ONE

THE PHYLOGENETIC RELATIONSHIPS OF THE PRASINOPHYCEAE AND THE PLEURASTROPHYCEAE INFERRED FROM ULTRASTRUCTURAL AND BIOCHEMICAL CHARACTERS

INTRODUCTION

Recent studies of green algal ultrastructure have provided new characters for hypotheses of evolutionary relationships among the major groups. Literature on the Chlorophyta has been summarized frequently, and in some cases new classifications have been proposed (Pickett-Heaps and Marchant 1972, Norris 1980, Moestrup 1982, Mattox and Stewart 1984, Melkonian 1984, O'Kelly and Floyd 1984, van den Hoek et al. 1988, Melkonian 1990a). One widely accepted classification is that of Mattox and Stewart (1984) who proposed five classes of the green algae: Charophyceae, Chlorophyceae, Pleurastrophyceae, Ulvophyceae, and the Micromonadophyceae (= Prasinophyceae).

Despite great attention to the Chlorophyta stimulated, in part, by their putative place in green plant evolution, few studies of their evolution have employed a rigorous phylogenetic (cladistic) approach to infer phylogenetic relationships. Using the ultrastructural characters discussed by Mattox and

Stewart (1984), Mishler and Churchill (1985) performed a cladistic analysis of the five classes of green algae and land plants. Sluiman (1985) also conducted a cladistic analysis of the major green algal groups (except pleurastrophytes) and land plants; however, this analysis was criticized by Theriot (1988) for inappropriate interpretation of the characters and for not finding all the most parsimonious cladograms. A preliminary cladistic analysis of ultrastructural and biochemical characters (Kantz et al. 1990) used a small data set of 12 characters and 11 taxa. At the class level the results were similar to those of Mishler and Churchill (1985). The Pleurastrophyceae was the sister clade to the Chlorophyceae. The Ulvophyceae was the sister clade to the Pleurastrophyceae + Chlorophyceae clade. The Pleurastrophyceae + Chlorophyceae + Ulvophyceae clade, the charophycean clade, and the prasinophycean taxa emerged from an unresolved basal node. Relationships within the Prasinophyceae were unresolved.

The following overview of the literature on the chlorophytan classes Pleurastrophyceae and

Prasinophyceae has provided a basis for a more inclusive cladistic analysis of ultrastructural, morphological, and biochemical data for the green algae. The analysis focuses on members of the Prasinophyceae (Micromonadophyceae) and the Pleurastrophyceae, to test the proposed monophyly of each of these classes.

Pleurastrophyceae

The Pleurastrophyceae includes coccoid, sarcinoid, and filamentous algae that were placed previously in different orders of the Chlorophyta (Mattox and Stewart 1984; Bold and Wynne 1985; Melkonian 1990d). Sexual reproduction is unknown among the Pleurastrophyceae. Asexual reproduction is by autospores or by biflagellate, flattened zoospores, which exhibit dorsiventral organization (Watson and Arnott 1973, Melkonian and Berns 1983).

The class is distinguished by possession of a counter-clockwise orientation of the basal bodies and an unusual mitotic spindle. During karyokinesis the

centrioles do not migrate to the cell poles, but instead remain in their interphase position in the same plane as the metaphase chromosomes, the so-called "metacentric" mitotic spindle (Molnar et al. 1975). Mitosis is closed (i.e. the nuclear envelope remains intact during nuclear division), and a centripetal furrow is associated with a phycoplast. Microtubules radiating from the centrioles "cup" the telophase nucleus.

Members of the Pleurastrophyceae occur in a variety of forms, despite the small number of genera (as many as eight, including Tetraselmis, a controversial taxon discussed below). Microthamnion, formerly of the Chaetophorales (Bold and Wynne 1985), is a branched filament with a holdfast found in freshwater habitats (Watson and Arnott 1973, Watson 1975). Pleurastrum is a branched filament without a holdfast commonly found in soil; it occasionally has been reported as a lichen phycobiont (Tupa 1974, Molnar et al. 1975, Melkonian 1981). Friedmannia is a sarcinoid soil alga formerly placed in the Chlorosarcinaceae (Deason et al. 1979, Melkonian and Berns 1983). Chlorosarcina is a

sarcinoid, freshwater alga (Deason and Floyd 1987), and Myrmecia is a sarcinoid alga with "typical" pleurastrophyte zoospore ultrastructure (Friedl, pers. comm.). Trebouxia, once placed in the Chlorococcales, is coccoid, and Pseudotrebouxia, traditionally placed in the Chlorosarcinales, is sarcinoid; both are found commonly as lichen phycobionts (Archibald 1975, Melkonian and Peveling 1988, Friedl 1989).

There is disagreement over the legitimacy of the genus Pseudotrebouxia vis-a-vis the genus Trebouxia. Archibald (1975) separated the sarcinoid Trebouxia species into the genus Pseudotrebouxia. Melkonian and Peveling (1988) examined Trebouxia and Pseudotrebouxia and found enough corroborative ultrastructural differences in the flagellar transition region to warrant continued recognition of both genera. A more extensive survey of species of Trebouxia and Pseudotrebouxia by Friedl (1989) showed that Trebouxia actually comprises several morphological types distinguished by ultrastructural morphology of the flagellar transition region, chloroplast, and pyrenoid, and by lifecycle details.

Pseudotrebouxia shares many ultrastructural features with one of the Trebouxia morphological types, prompting Friedl (1989) to merge Pseudotrebouxia into Trebouxia. None of these alternative hypotheses has been corroborated by cladistic analysis.

Taxonomy. The Pleurastrophyceae sensu Mattox and Stewart (1984) comprises two orders: the Tetraselmidales and the Pleurastrales (Table 1.1). The Tetraselmidales contains a single genus, the marine flagellate Tetraselmis, which traditionally is considered a prasinophyte alga (Norris 1980). The similarities of cell division between Pleurastrum and Tetraselmis (Molnar et al. 1975) prompted the inclusion of Tetraselmis in the Pleurastrophyceae (Mattox and Stewart 1984). They considered the Pleurastrophyceae to be the sister group to the Chlorophyceae because both classes possess a phycoplast.

Sluiman (1985) rejected the Tetraselmidales and considered the Pleurastrales to be a lineage within the Ulvophyceae, because both classes possess counter-clockwise basal body orientation. Melkonian (1990d) also rejected the Tetraselmidales and

Table 1.1. A comparison of the pleurastrophyte classification of Mattox and Stewart (1984) with that of Melkonian (1990).

Classification of Mattox and Stewart (1984)

Class Pleurastrophyceae
- Order Tetraselmidales
  - Genus Tetraselmis
- Order Pleurastrales
  - Genera Pleurastrum, Microthamnion, Pseudotrebouxia, Trebouxia, Friedmannia

Other taxa included in the Pleurastrales sensu Mattox and Stewart (Deason 1989)
- Chlorosarcina
- Myrmecia

Classification of Melkonian (1990)

Class Chlorophyceae (?)
- Order Microthamniales
  - Genera Pleurastrum, Microthamnion, Pseudotrebouxia, Trebouxia, Friedmannia

included Tetraselmis in the Chlorodendrales of the Prasinophyceae (Table 1.1). In addition, he prefered the ordinal designation Microthamniales, which he considered to be an order of "uncertain affinity," though likely a separate lineage of the Chlorophyceae.

Prasinophyceae

The Prasinophyceae is a class of unicellular flagellated algae that contain chlorophylls a and b, store starch in their chloroplasts, and many are covered with a layer of organic scales (Norris 1980). Most taxa are marine, though some are found in brackish and freshwater habitats. They are common in temperate and colder regions of both hemispheres, and are a prominent part of the marine phytoplankton (Leadbetter 1974, Manton 1977, Moestrup 1979, McFadden et al. 1986). Members of the Mamiellales (Mamiella, Mantoniella, Micromonas, Dolichomastix) are abundant in the picoplankton (Johnson and Sieburth 1982), and may constitute as many as 10% of the taxa in the deep photic zone (Hoepffener and Haas

1990). Freshwater prasinophytes (e.g. Tetraselmis cordiformis and Mesostigma viride) may form plankton blooms during the cold season (Melkonian 1990b). One prasinophyte species, Tetraselmis convolutae, is an extracellular endosymbiont of the flatworm Convoluta roscoffensis (Provasoli et al. 1968, Holligan and Gooday 1975).

Sexual reproduction is unknown for the majority of prasinophyte taxa, although one species of Nephroselmis is reportedly isogamous and heterothallic, with zygotic meiosis (Suda et al. 1989). The meiosis is unusual in that the first division occurs within the zygote cell wall and results in two biflagellate daughter cells. After the release of the daughter cells from the zygote wall, each undergoes the second meiotic division resulting in the production of a total of four haploid biflagellates.

Asexual reproduction in the Prasinophyceae is by mitosis and cytokinesis. In a few taxa an asexual resting cyst, called a phycoma (plural = phycomata), is formed, and may help the organism survive adverse conditions (Belcher 1966). The phycoma grows much

larger than the flagellated cells, and the number of cellular organelles increases by division. The cell contents eventually divide into numerous motile cells (Parke and Hartog-Adams 1965, Parke 1966, Parke et al. 1978). In coastal regions between Oregon and British Columbia the phycoma stage of species of Halosphaera and Pterosperma becomes prevalent in field collections in late December, and motile stages are released in early February (the late Maurice Dube, pers. comm. 1988). The phycoma stage in species of Halosphaera, Pachysphaera, and Pterosperma has a bilayered cell wall; the outer layer of the cell wall may be composed of sporopollenin (Melkonian 1990b).

Fossil phycomata are found in strata as late as the Recent period and as early as 900-1000 million years ago (MYA) in the late Precambrian (Loeblich 1974, Tappan 1980). The fossil genus Tasmanites is found in Ordovician (500 MYA) strata and resembles the phycoma stages of the extant prasinophycean genera Pterosperma and Pachysphaera (Tappan 1980). Fossil prasinophytes that resemble present day taxa

also have been found in Silurian (430 MYA) strata (Colbath 1983).

Taxonomy. Chadefaud (1941, 1960) distinguished certain green flagellates from the rest of the green algae because they possess a flagellar pit or groove, their Golgi bodies are in a parabasal position, and some taxa may have muciferous bodies or ejectosomes. Manton and Parke (1960) using electron microscopy discovered that some of the putatively wall-less flagellates discussed by Chadefaud were covered with organic scales.

Based upon these ultrastructural observations, in addition to his own studies, Christensen (1962) established the class Prasinophyceae to include phytoflagellates with a pyramidal or globular cell type, and a covering of non-mineralized organic scales on the cell body and flagella instead of a cell wall. Moestrup and Throndsen (1988) considered the Prasinophyceae to be a natural group, and provide a formal classification of the scale bearing forms. Melkonian (1990b) considered the Prasinophyceae monophyletic after the exclusion of the "problematic"

genera Pedinomonas and Monomastix; Pedinomonas is treated separately by him in the Pedinomonadales, a group with "uncertain affinity" (Melkonian 1990c).

Christensen (1962) originally included taxa with dorsiventral cells (viz. Mesostigma, Micromonas, Mantoniella, Nephroselmis, and Pedinomonas) in a second class Loxophyceae, but later (Christensen 1966) included only Micromonas and Pedinomonas. Subsequently, many workers have not recognized the Loxophyceae and have placed its taxa in the Prasinophyceae (Norris 1980, Mattox and Stewart 1984, Melkonian 1990b). Mattox and Stewart (1984) combined the taxa of the Loxophyceae and the Prasinophyceae (excluding Tetraselmis) as the Micromonadophyceae, but stated that the classification was weak and likely not a natural grouping. Moestrup (1982) recognized four genera as possible members of the Loxophyceae: Monomastix, Pedinomonas, Scourfieldia, and Micromonas. These genera all lack scales on the flagella, and Pedinomonas, Scourfieldia, and Micromonas lack them on the cell bodies, as well.

Moestrup (1991) presented a classification of some scaleless forms, called the Pedinophyceae, which

included Pedinomonas and a new genus Resultor (Table 1.2). Despite similarities between the Loxophyceae sensu Christensen (1966) and the Pedinophyceae sensu Moestrup (1991), Moestrup avoided the name Loxophyceae to prevent any confusion with older concepts of the Loxophyceae. Because the pedinophytes are small and uniflagellate (although they possess two basal bodies), with a counter-clockwise basal body orientation, a closed mitotic spindle, a persistant telophase spindle, and an eyespot that divides during cytokinesis, Moestrup considered the group to be an ancient divergence from the green algae, distinct from the prasinophyte lineage. Melkonian (1990c) also considered Pedinomonas to be distinct from the prasinophytes, and to be most closely related to the Ulvophyceae based on ultrastructural features of the flagellar apparatus.

Melkonian (1990b) distinguishes the Prasinophyceae with five characters (Table 1.2):

1) possess a covering (on the cell body and flagella) of non-mineralized organic scales,
2) possess parallel basal bodies,

3) bear tubular flagellar scales in two opposite rows,
4) have flagella that emerge from a groove or pit on the cell body,
5) possess parabasal golgi bodies lying close to the basal bodies.

Melkonian (1990b) stated that these features support monophyly for the class, despite the diversity of cell shapes, flagella number, karyo- and cytokinetic mechanisms, photosynthetic pigments, and storage products (Norris 1980, 1982).

Melkonian (1990b) recognized 13 genera of Prasinophytes, excluding Pedinomonas and Monomastix, in four orders (Table 1.2). Taxa in the Mamiellales (Moestrup 1984) possess a single layer of spiderweb-shaped scales on the cell body and flagella. The order has both uniflagellate (Micromonas and Mantoniella) and biflagellate genera (Mamiella and Dolichomastix), and the flagella are attached laterally. During swimming flagella are trailing and the undulating waves pass from base to tip. Mamiellales have only two flagellar roots, which are

Table 1.2. A comparison of the prasinophyte classification of Melkonian (1990) with that of Moestrup and Throndsen (1988) and Moestrup (1991).

Classification of Melkonian (1990)

Class Prasinophyceae
- **Order Mamiellales**
  - **Family Mamiellaceae**
    - **Genera** *Mamiella*, *Dolichomastix*, *Mantoniella*
  - **Family Micromonadaceae (?)**
    - **Genus** *Micromonas*
- **Order Pseudoscourfieldiales**
  - **Family Pseudoscourfieldiaceae**
    - **Genus** *Pseudoscourfieldia*
  - **Family Nephroselmidaceae**
    - **Genus** *Nephroselmis*
- **Order Chlorodendrales**
  - **Family Chlorodendraceae**
    - **Genera** *Tetraselmis*, *Scherffelia*
- **Order Pyramimonadales**
  - **Family Pterospermataceae**
    - **Genera** *Pterosperma*, *Pachysphaera*
  - **Family Pyramimonadaceae**
    - **Genera** *Pyramimonas*, *Halosphaera*
  - **Family Mesostigmataceae**
    - **Genus** *Mesostigma*

Order of uncertain affinity
- **Order Pedinomonadales**
  - **Genus** *Pedinomonas*

Classification of Moestrup and Throndsen (1988) and Moestrup (1991)

Class Prasinophyceae
- **Order Mamiellales**
  - **Family Mamiellaceae**
    - **Genera** *Mamiella*, *Mantoniella*, *Bathycoccus*, *Dolichomastix*, *Micromonas* (?)
- **Order Chlorodendrales**
  - **Family Halosphaeraceae**
    - **Genera** *Halosphaera*, *Pyramimonas*, *Pterosperma*, *Pachysphaera*, *Cymbomonas*, *Prasinochloris*
  - **Family Mesostimataceae**
    - **Genus** *Mesostigma*
  - **Family Chlorodendraceae**
    - **Genera** *Nephroselmis*, *Tetraselmis*, *Prasinocladus*, *Scherffelia*, *Pseudoscourfieldia*
- **Order Scourfieldiales**
  - **Family Scourfieldiaceae**
    - **Genus** *Scourfieldia*

Class Pedinophyceae
- **Order Pedinomonadales**
  - **Family Pedinomonadaceae**
    - **Genera** *Pedinomonas*, *Resultor*

associated with a single basal body. Melkonian (1984) considered the Mamiellales to have the most "primitive" characters of any order in the Chlorophyta.

The Pseudoscourfieldiales includes algae that have two layers of scales on the flagella. The under-layer scales are pentagonal and upper-layer scales are rod-shaped. In Pseudoscourfieldia both types of scales also cover the cell body. In Nephroselmis rod-shaped scales are absent on the cell body and are replaced by stellate scales. Cells are biflagellate with flagella of unequal length. During swimming at least one flagellum trails, with undulations passing from base to tip.

The members of the Chlorodendrales (Table 1.2), Tetraselmis and Scherfellia, are quadriflagellate and the flagella beat in two pairs in a breast stroke fashion (Melkonian and Preisig 1986). The flagellar root is cruciate and has two large system II fibers (rhizoplasts). Members possess a collapsing spindle during telophase and a phycoplast. The centrioles are metacentric (Mattox and Stewart 1984), lying in the same plane as the metaphase chromosomes, similar

to division in the Pleurastrophyceae (Mattox and Stewart 1984). Although Mattox and Stewart (1984) placed this group in the Pleurastrophyceae, Melkonian (1990d) retained it in the Prasinophyceae because of the presence of pentagonal and rod-shaped flagellar scales similar to those in the Pseudoscourfieldiales. The cell body is covered by a theca formed by the fusion of golgi-derived scales, which resemble the stellate scales of Nephroselmis.

Members of the Pyramimonadales have three layers of scales on the body and two on the flagella. Melkonian (1990b) recognized three families of the Pyramimonadales: the Pyramimonadaceae, the Pterospermataceae, and the Mesostigmataceae. In the Pyramimonadaceae the outermost scale layer of the cell body consists of "crown" (basket-shaped) scales, the middle layer of box-shaped scales, and the inner layer of square or pentagonal scales. The middle layer in the Pterospermataceae consists of spiderweb-shaped scales, and in the Mesostigmataceae, of naviculoid scales. The flagella are covered by an inner layer of square or pentagonal scales and an outer layer of limulus-shaped scales, which resemble

spiderweb-shaped scales (McFadden et al. 1986) and might be homologous to the middle layer of scales on the body and the spiderweb-shaped scales of the Mamiellales. The Pyramimonadales possess a system II fiber and (with the exception of Mesostigma) have a transitional helix or coiled fiber in the flagellar transition region (Moestrup 1982; Melkonian 1984).

Mesostigma, the only member of the Mesostigmataceae, is biflagellate and the flagella beat in a "breast stroke" fashion. It possesses a cruciate flagellar root system and a multi-layered structure (Rogers et al. 1981; Melkonian 1989).

The Pyramimonadaceae include quadri- or octaflagellates with a cruciate flagellar root system, and the flagella beat in a "breast stroke" fashion. Pyramimonas has been divided into three subgenera (McFadden et al. 1986), and trichocysts are found in one of the sub-genera. Halosphaera has a phycoma stage which gives rise to a Pyramimonas-like motile stage (Manton et al. 1963). Halosphaera minor has been reported to possess a multilayered structure associated with the flagellar root (Hori et al. 1985), but this culture has been lost and the feature

cannot be varified (Charles J. O'Kelly, Massey University, New Zealand, pers comm. 1991).

The flagellar root system of the Pterospermataceae resembles that of Halosphaera (Melkonian 1990b), but differs from Pyramimonas (Parke et al. 1978); however, the mode of swimming is "backward" with undulating movement of the flagella. As in Halosphaera, members of this group form phycomata.

MATERIALS AND METHODS

A data set of 44 characters (Table 1.3) for 37 taxa of green algae and three outgroups was compiled from the primary literature. References may be found in the review papers of Norris (1980), Moestrup (1982), Mattox and Stewart (1984), Melkonian (1984), O'Kelly and Floyd (1984), van den Hoek et al. (1988), and Melkonian (1990a-d). States of all multistate characters were coded as unordered (Tables 1.3 and 1.4). Data were analyzed cladistically with the heuristic tree search algorithms contained in the

Table 1.3. List of 44 characters for 40 taxa (see text for character descriptions and references). Symbols for the character states are in parentheses.

Flagellar characters
1. Flagellar root system symmetry (0=asymmetric 1=cruciate)
2. Clockwise basal body orientation (0=absent 1=present)
3. Multi-layered Structure (MLS) (0=absent 1=present)
4. Striated Microtubule Associated Component (SMAC) (0=absent 1=present)
5. Rhizoplast (System II fiber) (0=absent 1=present)
6. Number of flagella (0=number of flagella)
7. Basal Body length (0=long 1=short)
8. Number of parts of stellate structure (0=stellate structure absent 1=1 part 2=2 parts)
9. Septum attached to flagellar membrane (0=not attached 1=attached)
10. Central dilation of septum (0=absent 1=present)
11. Septum intercalated within stellate structure (0=not intercalated 1=intercalated)
12. Additional septa present (0=absent 1=present)
13. Flagellar pit (0=absent 1=present)
14. Flagellar beat (0=undulating flagella 1=breast stroke)
15. Transitional helix (0=absent 1=present)

Cell covering
16. Flagellar "spider-web" scales (0=absent 1=present)
17. Flagellar pentagonal/square scales (0=absent 1=present)
18. Flagellar "rod" scales (0=absent 1=present)
19. Flagellar tubular hair scales (0=absent 1=present)
20. Flagellar limulus scales (0=absent 1=present)
21. Cell body "spider-web" scales (0=absent 1=present)
22. Cell body pentagonal/square scales (0=absent 1=present)
23. Cell body stellate scales (0=absent 1=present and unfused 2=present and fused)
24. Cell body rod-shaped scales (0=absent 1=present)
25. Cell body crown scales (0=absent 1=present)
26. Cell body box scales (0=absent 1=present)
27. Cell body naviculoid scales (0=absent 1=present)
28. Cell wall on motile stage (0=absent 1=present)

Mitotic characters
29. Closed or open spindle (0=closed 1=open)
30. Persistent or collapsing spindle (0=persistent 1=collapsing)
31. Phragmoplast / Cell plate (0=absent 1=present)
32. Phycoplast (0=absent 1=present)
33. Metacentric mitotic spindle apparatus (0=absent 1=present)
34. Microtubules cup nucleus during mitosis (0=absent 1=present)

Biochemistry
35. Glycollate oxidase (0=absent 1=present)
36. Size of LHPC (0=small 1=large)
37. Mg 2,4-divinylphaeoporphyrin a5 monomethyl ester (0=absent 1=present)
38. Prasinoxanthin (Xanthophyll K) (0=absent 1=present)
39. Xanthophylls K1, K2 (0=absent 1=present)
40. Chlorophyll b (0=absent 1=present)

Morphology
41. Gross Morphology (1=coccoid 2=sarcinoid 3=filamentous 4=flagellate 5=parenchymatous)
42. Pyrenoid invaginations (0=absent 1=thylakoid intrusion 2=cytoplasmic intrusion)
43. Phycoma stage (0=absent 1=present)
44. Double membrane bound plastid (0=absent 1=present)

Table 1.4. Data matrix of 44 characters for 40 taxa (see Table 1.3 and text for character descriptions and references).

	1	2	3	4
	12345678901234 5	6789012345678	901234	567890 1234
Chlorophyceae				
Chlamydomonas eugametos	1101021211100 10	0000000000001	010100	000001 4001
Chlamydomonas moewusii	1101021211100 10	0000000000001	010100	000001 4001
Chlamydomonas reinhardtii	1101021211100 10	0000000000001	010100	000001 4001
Asteromonas	1101021211100 10	0000000000000	010100	000001 4001
Nanochlorum	??0???1???????	????????????	010100	000001 1?01
Chlorella	??0???1???????	????????????	010100	000001 1?01
Pleurastrophyceae				
Pseudotrebouxia	1001121200100 10	0000000000000	010111	000001 2001
Pleurastrum	1001121200100 10	0000000000000	010111	000001 3001
Friedmannia	1001121200100 10	0000000000000	010111	000001 2001
Microthamnion	1001121200100 10	0000000000000	010111	000001 3001
Chlorosarcina	1001121100100 10	0000000000000	0?0111	000001 2001
Myrmecia	1001121200100 10	0000000000000	??????	000001 2?01
Prasinophyceae				
Mamiella	0?0012?1????0100	1001010000000	??????	?11171 4001
Dolichomastix	0?0012?1????0100	1001010000000	??????	?????1 4001
Mantoniella	0?0012017000100	1001010000000	??????	011101 4001
Micromonas	0?00?1??????100	0007000000000	??????	011101 4001
Pseudoscourfieldia	0?0012?20111100	0111001010000	??0?00	0?1??1 4201
Nephroselmis	0?0012?20111100	0111001100000	??0?00	001001 4?01
Tetraselmis carteriiformis	100014?20010110	0111007200000	010111	000001 4201
Tetraselmis levis	100014?20010110	0111007200000	010111	000001 4201
Scherffelia	100014?20010110	0111007200000	?10111	??0001 4?01
Pterosperma	1007?14??????101	0101111001000	??????	??1011 4?11
Pachysphaera	1007?14??????101	0101111001000	??????	??1011 4?11
Pyramimonas	1000140?????0111	0101101001100	0?0?00	0?1011 4101
Halosphaera	1010140?????0111	0101101001100	??????	??0001 4?11
Mesostigma	101??2??????110	0100101001010	??????	??0001 4?01
Monomastix	??0?11?1?000??0	0007100000000	??????	??0001 4?01
Scourfieldia	??0?12017000??0	0007000000000	??????	?????1 4?01
Pedinomonas minor	1001111100101 00	0007000000000	000?00	000001 4101
Pedinomonas tuberculata	1001111100101 00	0007000000000	000?00	000001 4101
Pedinomonas minutissima	??????????????0?	0007000000000	??????	?????1 4?01
Ulvophyceae				
Bryopsis	1001121100100 10	0000000000000	000?00	000011 ??01
Enteromorpha	1001121100100 10	0000000000000	000?00	000011 3?01
Charophyceae and Land Plants				
Glycine	??7000017??0???	????????????	101000	100001 5?01
Zamia	0010000177?0???	????????????	101000	100001 5?01
Equisetum	0010020177?0???	????????????	101000	100001 5?01
Klebsormidium	0010020177?0010	0007000000007	101000	100001 3?01
Outgroups				
Phaeodactylum	???????0??????	????700000007	??0000	??0000 1?00
Saccharomyces	???????0??????	????????????	??0000	?????0 1?00
Costaria	000002?0????0??	0000000000000	??0000	??0000 5?00

software package Phylogenetic Analysis Using Parsimony (PAUP v. 3.0q, Swofford 1989). The search was conducted 100 times with the taxa added in random sequence to increase the probability of finding all most parsimonious trees. The search algorithm Tree-Bisection-Reconnection (TBR) was used to find the most parsimonious trees. Hennig86 (ver. 1.5, Farris 1988) also was used with the commands mhennig* (tree-searching) and bb* (branch-breaking). Cladogram lengths and topologies resulting from the two programs were identical. Alternative rooting experiments were conducted by enforcing topological constraints in PAUP and in Hennig86. The lengths of the alternative topologies were then compared to examine the relative support for various hypotheses.

CHARACTER STATE ANALYSIS

A list of characters and the character states for each taxon are provided in Table 1.3 and Table 1.4.

Flagellar Characters (characters 1-15)

Ultrastructural features of the flagellar apparatus play an important part in modern classifications of the green algae. The symmetry of the flagellar root system (character 1) is an important character used to distinguish green algae at the class level (Mattox and Stewart 1984). The flagellar root may be aymmetric or symmetric (cruciate), and the cruciate flagellar root system (character 1) is considered a synapomorphy for the Ulvophyceae, Pleurastrophyceae, and Chlorophyceae (Mattox and Stewart 1984), but some prasinophytes possess a cruciate flagellar root system (Melkonian 1990b). Taxa with a cruciate flagellar apparatus have their basal bodies arranged in a clockwise or a counter-clockwise fashion (some taxa not included in the data matrix have directly opposed basal bodies). The clockwise arrangement (character 2) distinguishes the Chlorophyceae sensu Mattox and Stewart (1984).

The multilayered structure (MLS), character 3, is considered characteristic of the Charophyceae (Mattox and Stewart 1984), but an MLS is also found in Mesostigma (Rogers et al. 1981; Melkonian, 1989),

and has been reported in Halosphaera (Hori et al. 1985).

Character 4, the striated microtubule associated component (SMAC) (Floyd et al. 1980), also known as the System I fibrous root (Melkonian, 1984), is found in the Chlorophyceae, the Pleurastrophyceae, and the Ulvophyceae (Melkonian, 1984).

The rhizoplast, or System II fibrous root (character 5), is found in four major groups of the algae (Chlorophyceae, Ulvophyceae, Pleurastrophyceae, and Prasinophyceae), but apparently is lacking in the Charophyceae (Mattox and Stewart, 1984). Tetraselmis and Pyramimonas both have two System II fibers (Melkonian, 1984).

The number of flagella (character 6) varies from one to two to four. Eight or more flagella may occur in some organisms (e.g., the Oedogoniales and one species of Pyramimonas), but these cases are generally regarded as derived conditions (Moestrup 1982, Melkonian 1984). The biflagellate condition is most common. There is much speculation over whether the primitive condition is uniflagellate (and bi- and quadriflagellates arose from successive doubling of

the flagellar apparatus, Melkonian, 1984); or biflagellate (with a reduction to the uniflagellate condition and a doubling to the quadriflagellate condition, Mattox and Stewart, 1984); or quadriflagellate (similar to Pyramimonas, with successive reductions to the bi- and uniflagellate condition, O'Kelly and Floyd 1984, Mattox and Stewart 1984).

The length of the basal body (character 7) is thought to have some taxonomic use. Most prasinophytes (except Pedinomonas) and charophytes have relatively long basal bodies (> 500 nm), and chlorophytes, ulvophytes and pleurastrophytes have relatively short basal bodies (300-400 nm, Melkonian, 1984).

The flagellar transition region (characters 8-12) is that region between the flagellar shaft and the basal body (Moestrup, 1982). Melkonian (1984) examined the flagellar transition region for many of the Prasinophytes. He states that at least 15 characters can be identified in the transition region, though he does not specifically list them. Instead, he recognizes several transition region

"types" corresponding to the arrangement of the characters in specific organisms (for example, the "Tetraselmis type," or the "Pedinomonas type"). If each type is included in a data matrix, rather than considering each character separately, then nearly every type becomes autapomorphic and, therefore, is of no value in a cladistic analysis. For this reason, each of the transition region types was divided into separate characters. Based upon Melkonian's discussion and diagrams five phylogenetically informative characters for the transition region were identified. A discussion of each follows.

The stellate structure (character 8), if present, is found either as a continuous cylinder throughout the length of the transition region, or in two parts, forming both proximal and distal cylinders (Melkonian, 1984). A transverse septum within the transition region may or may not: be attached to the flagellar membrane (character 9); have a central dilation (character 10); and be intercalated within the stellate structure (character 11). Because characters 10-12 refer to features of the transverse septum, those organisms without a septum (e.g.,

Mesostigma, Pyramimonas, and the Charophyceae) are coded as "unknown" for those characters. Nephroselmis and Pseudoscourfieldia possess two transverse septa: an intercalated septum, and an additional septum distal to the stellate structure (character 12) (Melkonian, 1984).

The presence of a flagellar pit (character 13) from which the flagella emerge, was a character originally used to distinguish the Prasinophyceae (Christensen 1966, Melkonian, 1990b). O'Kelly and Floyd (1984) have speculated that the flagellar pit is derived from a feeding apparatus present in the protozoan that first acquired a green chloroplast; thus, the presence of a flagellar pit may be a symplesiomorphy.

The flagellar beat (character 14) in many green algae is a "breast stroke," but in some prasinophytes the flagella trail the cell body (even if the flagella are inserted apically) and beat in an "undulating" fashion (Melkonian 1990b).

The transitional helix (character 15) is a coiled structure found in the flagellar transition region in Pyramimonas, Halosphaera, Pterosperma, and

Pachysphaera (Moestrup 1982; Melkonian 1984; Melkonian 1990b).

Cell Covering (characters 16-28)

The flagella of the Prasinophytes may be naked or covered with one or two layers of golgi-derived scales (characters 16-20), and each layer has only one type of scale. If one layer is present the scale generally has a "spider-web" shape. If two layers are present then the layer closest to the cell membrane is generally pentagonal. The outer layer of scales may be spider-web-shaped (e.g. Pyramimonas) or rod-shaped (e.g. Tetraselmis), and additional scale types may be present (Norris, 1980; Moestrup,1982; Melkonian, 1984). The Charophyceae commonly have a single layer of small pentagonal or square-shaped scales on their flagella and cell body, though they are lacking in Klebsormidium (Melkonian, 1984; Moestrup 1982). The scales on the flagella often differ morphologically from the scales found on the cell body; therefore, flagellar scale characters have been treated as independent of cell body scale characters.

Characters 21-28 refer to the covering of the cell body of the motile stage. Many prasinophytes are covered by one or more layers of golgi-derived scales, and each layer is composed of a single type of scale (Norris 1980, Moestrup 1982, Melkonian 1984, Melkonian 1990). Rather than possessing scales, an organism may possess a theca of fused scales, a cell wall, or may be naked. The theca of Tetraselmis apparently arises through the extracellular fusion of golgi-derived scales that resemble the scales of Nephroselmis and Pseudoscourfieldia (Domozych et al., 1981). The motile cells of Chlamydomonas possess a cell wall of glycoprotein which is unlikely to be homologous to the scales of prasinophytes (Melkonian 1990b). There is no separate character in the data matrix for the naked condition; absence of the other cell coverings implies a naked cell body.

Mitotic Characters (characters 29-34)

Features of karyo- and cytokinesis have been used to distinguish green algal classes (Pickett-Heaps 1972, Mattox and Stewart 1984, O'Kelly and

Floyd 1984). The mitotic spindle may be open or closed (character 29), and it may persist or collapse during telophase (character 30). The phragmoplast and cell plate (character 31) are found in the Charophyceae and land plants, and a phycoplast (character 32) is found in the Chlorophyceae and Pleurastrophyceae. The metacentric mitotic spindle (character 33) and microtubules that cup the nucleus (character 34) distinguish the Pleurastrophyceae sensu Mattox and Stewart (1984), which includes Tetraselmis.

Biochemistry (characters 35-40)

Glycolate oxidase (character 35), an enzyme of photorespiration, is found in the Charophyceae and land plants, whereas glycolate dehydrogenase is found in other groups of green algae (Frederick et al. 1973).

Character 36 refers to the presence of a large 54,000-55,000 dalton light harvesting complex (LHC) found in Mantoniella and Micromonas, compared to the smaller 25,000-35,000 dalton LHC found in all other green algae so far examined (Fawley et al. 1986).

The presence of the pigment magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester (character 37) is of taxonomic interest (Ricketts 1970, Rowan 1989). This pigment recently has been shown to have the same absorption spectrum as chlorophyll c (Wilhelm et al., 1986). Those organisims with Mg 2,4-divinylphaeoporphyrin a_5 monomethyl ester fall into two groups: those with prasinoxanthin (character 38), and those with xanthophylls K1 and K2 (character 39) (Ricketts 1970, Foss et al. 1984).

The Chlorophyta is distinguished by the presence of a chloroplast with the accessory pigment chlorophyll b (character 40) (Bold and Wynne 1985, Rowan 1989). It is included in the data matrix as a character supporting monophyly of the ingroup.

Morphology (characters 42-45)

The morphology of the dominant stage in the life cycle (character 42) is not considered useful at the class level (Mattox and Stewart 1984, O'Kelly and Floyd 1984), but it has been used for classification at lower taxonomic levels.

Intrusions of thylakoids or cytoplasm into the pyrenoid (character 43) occur in Pedinomonas, Pyramimonas, and Tetraselmis (Norris 1980, Moestrup 1991).

Halosphaera, Pachysphaera, and Pterosperma have a phycoma stage, a resistant cyst, (character 44) in their life cycle (Norris 1980, Moestrup 1982, Moestrup 1984).

The Chlorophyta possess a chloroplast with two surrounding membranes (character 45), whereas the outgroup taxa lack a plastid or have more than two surrounding membranes (Bold and Wynne 1985).

RESULTS AND DISCUSSION

All Taxa

Analysis of the 44 characters and 40 taxa resulted in 737 equally most parsimonious cladograms. For the cladograms (length = 81 steps, CI = 0.64, and RI = 0.87) a strict consensus tree was constructed (Fig. 1.1). The consensus tree shows a clade of pleurastrophytes + chlorophytes, with relationships among the taxa within this clade unresolved. The

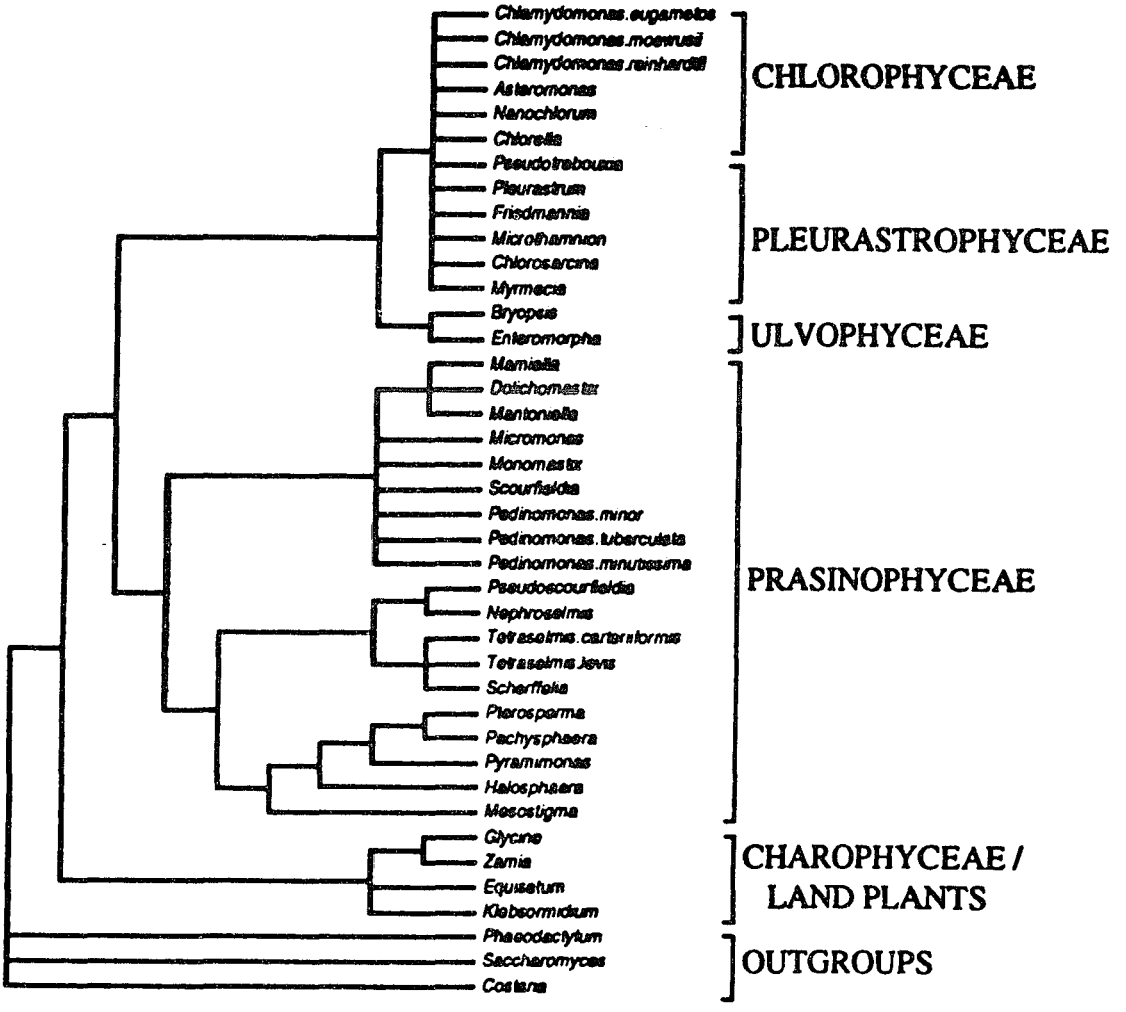

Figure 1.1. Strict consensus tree of the 737 cladograms generated from the complete data set of 44 characters and 40 taxa. The tree lengths were 81 steps, CI = 0.64, RI = 0.87.

Ulvophyceae form a sister clade to the pleurastrophyte + chlorophyte clade, and the Prasinophyceae form a monophyletic clade basal to the ulvophyte + chlorophyte + pleurastrophyte clade. The charophyte + land plant clade is basal to the rest of the green algae.

The large number of cladograms appears to be due to a few taxa with many unknown character states (Nanochlorum, Chlorella, Pedinomonas minutissima, Glycine, Zamia, Equisetum, and Saccharomyces); sequentially deleting these taxa from the analysis results in a single parsimonious cladogram (Fig. 1.2). These deleted taxa have 20 or more (>45%) character states coded as unknown. All these taxa, except P. minutissima, lack a motile stage, so that character states of the motile stage were coded as unknown. With the deletion of these taxa, characters 24 (rod-shaped scales on the cell body), 27 (naviculoid scales on the cell body), 29 (the open spindle), 31 (the phragmoplast), and 35 (glycolate oxidase) become uninformative and were excluded from the analysis.

Reduced Data Set

The single most parsimonious tree resulting from the analysis of the reduced data set has a length of 73 steps, a consistency index of 0.63 and a retention index of 0.85 (Fig. 1.2). The Charophyceae is again shown to be a basal lineage of the green algae. The pleurastrophycean taxa are now resolved as a clade sister to a chlorophycean clade. The Ulvophyceae is once again sister to the Pleurastrophyceae plus Chlorophyceae clade. Although the arrangement of these three classes is consistent with the hypotheses of Mishler and Churchill (1985) and Mattox and Stewart (1984), it is not consistent with the hypothesis of Sluiman (1985), who considered the Pleurastrophyceae to be a lineage within the Ulvophyceae.

Relationships within the Pleurastrophyceae are unclear. The four sarcinoid genera Myrmecia, Chlorosarcina, Friedmannia, and Pseudotrebouxia form a clade but relationships among the genera are unresolved. The clade of sarcinoid taxa and the two filamentous taxa, Pleurastrum and Microthamnion, form a trichotomy.

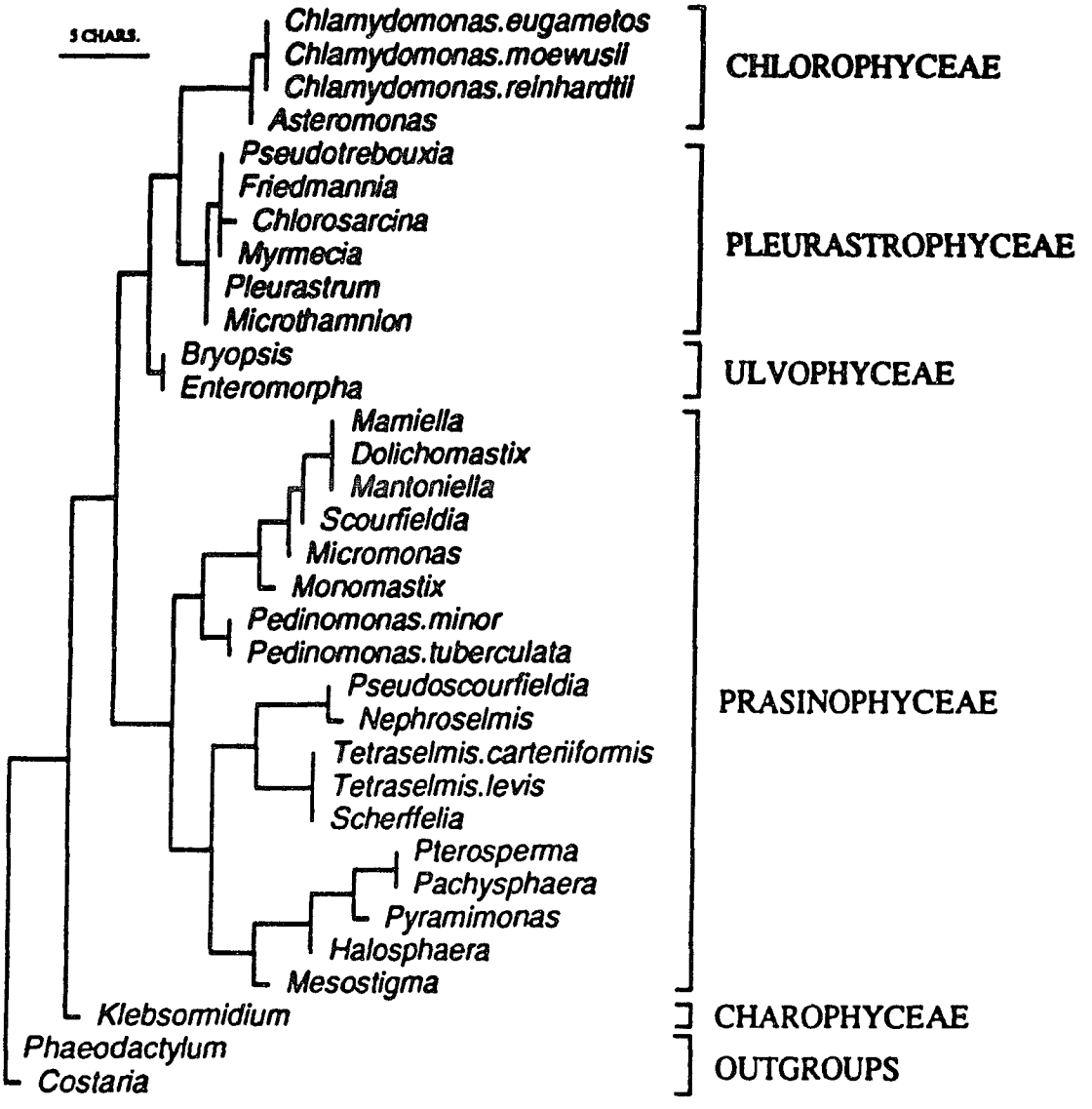

Figure 1.2. The single parsimonious cladogram resulting after the deletion of 7 taxa which had >20 characters coded as unknown. The cladogram length is 73 steps, CI = 0.63, RI = 0.85.

As in the complete analysis, the Prasinophyceae form a clade basal to the clade of pleurastrophytes, chlorophytes, and ulvophytes. The prasinophycean taxa comprise two major clades. The taxa in one of the clades correspond to the Chlorodendrales sensu Moestrup and Throndsen (1988) and to the Chlorodendrales, Pseudoscourfieldiales, and Pyramimonadales sensu Melkonian (1990b). The other clade contains taxa of the Mamiellales and the Pedinomonadales sensu Melkonian (1990c) and of the Mamiellales, Scourfieldiales, and Pedinophyceae sensu Moestrup (1991).

The genus Tetraselmis is placed in the Pleurastrophyceae by Mattox and Stewart (1984) due to the presence of a metacentric mitotic spindle and the cupping microtubules, but it is placed in the Prasinophyceae by Moestrup and Throndsen (1988) and Melkonian (1990b) due to features of its flagellar scales and its flagellar apparatus. The cladistic analyses of the morphological data in both cases show that Tetraselmis is more closely related to prasinophytes than to the pleurastrophytes (sensu Mattox and Stewart 1984). Thus, the metacentric

spindle and the cupping microtubules are homoplastic in this analysis. These results are consistent with the speculations of Melkonian (1984) that the evolution of the metacentric spindle might be related to the evolution of the theca, and that the presence of the metacentric spindle in Tetraselmis and in the Pleurastrophyceae may be a convergence due to the separate origin of the theca by scale fusion in Tetraselmis. However, the relatively large number of characters for the flagellar apparatus and cell covering available, compared to the lower number of mitotic characters, may be biasing the results in favor of the hypotheses of Melkonian (1990b) and Moestrup and Throndsen (1988).

Melkonian (1990c) treats the Pedinomonadales as a group with "uncertain affinities," probably related to the Ulvophyceae based primarily on similarities of the flagellar root and the flagellar transition region, or possibly to the Prasinophyceae (especially the Mamiellales) based on the type of flagellar insertion and flagellar beat, the parabasal golgi apparatus, and the persistent telophase spindle (Melkonian 1984, 1990c). Cladistic analysis shows

that Pedinomonas is more closely related to the Mamiellales than to the Ulvophyceaee. The ultrastructural similarities between Pedinomonas and the Ulvophyceae are apparently symplesiomorphic.

The Ancestral Flagellate

The characteristics of the ancestral green flagellate have been controversial. Hypotheses vary about whether the ancestral green flagellate most closely resembled Pedinomonas (Moestrup 1991), members of the Mamiellales (Melkonian 1990b), or Pyramimonas or Halosphaera (O'Kelly and Floyd 1984, Floyd and O'Kelly 1990).

Rerooting the cladogram at the different prasinophycean lineages is one method of testing the relative character support for the various hypotheses. If the cladogram is rooted at the Mamiellales the tree length increases from 73 steps to 78 steps. Rerooting at the Pedinomonadales produces a tree with a length of 79 steps. And rerooting at the Pyramimonadales produces a tree with a length of 80 steps. Thus, the hypothesis that the Mamiellales is the basal lineage of the green algae

has the most support, albeit by few steps, of the three hypotheses. The hypothesis of a basal Pyramimonadales is the least well supported of the hypotheses.

Another way to test these alternative hypotheses is by reconstructing the likely ancestor of the green algae (Table 1.5) in a method similar to that used by Mishler and Churchill (1985) to reconstruct the likely archetype for land plants. This reconstructed ancestor is strictly hypothetical because it is based on a parsimonious interpretation of characters of extant taxa, without incorporation of information from the fossil record (Mishler and Churchill 1985). Nonetheless, such evolutionary scenarios can be formulated in a logical, rigorous, and non-circular way using cladograms and the information they provide on character transformations (Eldredge 1979).

The character states of the basal node suggest that the ancestral green alga was biflagellate with an asymmetric flagellar root and no rhizoplast (characters 1, 5, and 6). Such an arrangement resembles that found in the Mamiellales and the charophycean algae. The stellate structure of the

Table 1.5. The character states of the basal node of the ingroup. Character numberings follow those in Table 1.3. The states for the terminal taxa Mamiella, Pedinomonas minor, and Pyramimonas are provided for comparison.

Character numbers	1 123456789012345	2 6789012345678	3 901234	4 567890	 1234
Basal node	000002010010010	0000000000000	?00000	?00001	3?01
Mamiella	0?00127?7?70100	1001010000000	??????	?11?71	4001
Pedinomonas minor	100111110010100	0007000000000	000?00	000001	4101
Pyramimonas	10001401???0111	0101101001100	0?0?00	0?1011	4101

flagellar transition region would be composed of one part in the hypothetical ancestor. A septum intercalated within the stellate structure, would not be attached to the membrane, and would lack a central dilation. No additional septa would be present (characters 8-12). The hypothetical arrangement of the stellate structure is most like that found in Pedinomonas or the Ulvophyceae. The ancestor would lack cell and flagellar covering (characters 16-28), and have a spindle persisting throughout mitosis (character 30). These features are also like those found in Pedinomonas and the Ulvophyceae. Thus, the results of the rerooting experiment and the reconstructed ancestor show support for either a Mamiellales-like ancestor or a Pedinomonas-like ancestor, but not for a Pyramimonas or Halosphaera-like ancestor.

CONCLUSION

A cladistic analysis of ultrastructural and biochemical data available for representatives of major groups of the green algae shows congruence at

the class level with prior cladistic analyses using fewer taxa and fewer characters (Mishler and Churchill 1985, Kantz et al. 1990). Within the Prasinophyceae and the Pleurastrophyceae the cladistic analysis shows much congruence with current taxonomic systems (Mattox and Stewart 1984, Moestrup and Throndsen 1988, Melkonian 1990b, Melkonian 1990d). Based upon this analysis the genus Pedinomonas is more closely related to the Mamiellales than to the Ulvophyceae. The ultrastructural data indicate the problematic genus Tetraselmis is more closely related to prasinophyte taxa than to pleurastrophyte taxa, which supports the views of Melkonian (1990b), but not Mattox and Stewart (1984).

Rerooting experiments and reconstruction of the ancestral node of the green algae support the hypotheses that the ancestral green flagellate was Pedinomonas-like or Mamiellales-like (Moestrup 1991, Melkonian 1990b), but provide less support for a Pyramimonadales-like ancestor (O'Kelly and Floyd 1984).

Despite the fact that this study is the most exhaustive phylogenetic analysis to date of the organismal data available for these taxa, the preponderance of characters for the flagellar apparatus and cell covering may be biasing the analysis, especially if there are structural and functional correlations of characters within these character systems. The relative weight accorded to these character systems by the large number of characters taken from them might overwhelm the phylogenetic signal from the other independent character systems. Also, with the type of characters considered, it is difficult to find any outgroup taxa that provide a complete or nearly complete array of characters comparable to the ingroup. Clearly, additional independent data sets are needed to test the hypotheses of chlorophyte evolution proposed in this study.

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CHAPTER TWO

THE PLEURASTROPHYCEAE AND MICROMONADOPHYCEAE: A CLADISTIC ANALYSIS OF NUCLEAR rRNA SEQUENCE DATA

October 1, 1992

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JOURNAL OF PHYCOLOGY
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Kantz, T.S., Theriot, E.C., Zimmer, E.A., & Chapman, R.L. 1990. The Pleurastrophyceae and Micromonadophyceae: A cladistic analysis of nuclear rRNA sequence data. J. Phycol. 26:711-721.

Thank you very much for your assistance.

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THE PLEURASTROPHYCEAE AND MICROMONADOPHYCEAE: A CLADISTIC ANALYSIS OF NUCLEAR rRNA SEQUENCE DATA[1]

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ABSTRACT

Partial sequences from the nuclear-encoded 18S and 26S ribosomal RNA molecules from representatives of the five classes of Chlorophyta sensu *Mattox and Stewart (1984) were analyzed cladistically in a study of the phylogenetic relationships among the Micromonadophyceae, Pleurastrophyceae, and other green algae. The sequence data indicate that the Micromonadophyceae (= Prasinophyceae) is not monophyletic but comprises at least three lineages occupying a basal position among the green algae. Though the Pleurastrophyceae and the Ulvophyceae both possess counter-clockwise basal body orientations, the sequence data indicate that the Pleurastrophyceae is the sister group to the Chlorophyceae. The molecular data alone do not resolve the monophyly of the Pleurastrophyceae or the Ulvophyceae; however, a combined data set of molecular and non-molecular characters support a monophyletic Pleurastrophyceae. Analyses with user-defined tree topologies and the bootstrap method of character resampling indicate that the relationships shown in the most parsimonious cladograms are well supported by the character data.*

Key index words: Chlorophyta; cladistics; Micromonadophyceae; phylogeny; Pleurastrophyceae; Prasinophyceae; rRNA sequencing; systematics

Comparative ultrastructure studies have led to many new hypotheses about green algal phylogeny and systematics (e.g. Floyd et al. 1972, Pickett-Heaps 1972, Pickett-Heaps and Marchant 1972, Stewart and Mattox 1975). Not all new classifications based on ultrastructural characters have been accepted, but the premise that ultrastructural characters, such as features of motile cell ultrastructure and cytokinesis, provide phylogenetically informative data is generally accepted.

A widely accepted classification system is that of Mattox and Stewart (1984), who recognize five classes of green algae: Chlorophyceae, Charophyceae, Ulvophyceae, Pleurastrophyceae, and Micromonadophyceae (= Prasinophyceae). Although Mattox and Stewart's (1984) proposal may approach a more natural evolutionary classification of green algae, they themselves call for further testing of their phylogenetic hypotheses.

Mishler and Churchill (1985) used many of the characters discussed by Mattox and Stewart (1984) in a cladistic analysis of green algae and land plants. At the class level, the cladistic analysis is closely congruent with the phylogenies proposed by Mattox and Stewart (1984) and O'Kelly and Floyd (1984b).

Our study focuses on two problematic classes: the Pleurastrophyceae and the Micromonadophyceae. The Pleurastrophyceae is a small but morphologically and ecologically diverse class that includes flagellated, coccoid, sarcinoid, and filamentous forms found in marine, freshwater, terrestrial, and lichenized habitats. Although members of the class are traditionally placed in separate orders of the Chlorophyta (*sensu* Bold and Wynne 1985), their ultrastructural similarities suggest a close phylogenetic relationship (Mattox and Stewart 1984, O'Kelly and Floyd 1984b, Sluiman 1989).

The Pleurastrophyceae *sensu* Mattox and Stewart (1984) is distinguished in part by a counter-clockwise orientation of the basal bodies, a metacentric mitotic spindle apparatus, and a collapsing telophase spindle. Its phylogenetic position is uncertain because it shares the collapsing telophase spindle with the Chlorophyceae and a counter-clockwise orientation of the basal bodies with the Ulvophyceae. In the Mattox and Stewart system the Pleurastrophyceae

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is a sister group of the Chlorophyceae, a relationship also indicated in Mishler and Churchill (1985). However, Sluiman (1989) reduced the Pleurastrophyceae from its class ranking to that of an order, the Pleurastrales, within the Ulvophyceae. Furthermore, he discounted the use of cytokinetic characters, contending they are inappropriate characters at the class level.

The Micromonadophyceae, a diverse group of green flagellates, is often regarded as the most primitive group of the green algae, possibly representing the ancestral stock from which the other classes arose (Norris 1980, Moestrup 1982, Mattox and Stewart 1984, Melkonian 1984, O'Kelly and Floyd 1984b, Round 1984). The Micromonadophyceae *sensu* Mattox and Stewart (1984) is distinguished by one character, a persistent interzonal mitotic spindle, which is likely primitive for the green algae. Mattox and Stewart (1984) themselves consider the "present necessity to place all the scaly or naked green flagellates with persistent interzonal spindles in a single class, Micromonadophyceae" to be the greatest weakness of their classification. They even suggest that the class may not be monophyletic, and that members of the class may, upon further study, be moved to other classes.

Not only are evolutionary relationships among the Micromonadophyceae and the other green algal classes unresolved, but within the Micromonadophyceae, relationships are uncertain. Many members of the class possess a cell and flagellar covering of golgi-derived scales, and much of the taxonomy of the group is focused on the number of scale layers and scale morphology (Norris 1980, Moestrup 1982, Pennick 1984). However, evolutionary schemes based on scale morphology often contradict those based on ultrastructural features (Norris 1980, Moestrup 1982, Melkonian 1984), possibly due to the lack of a cladistic analysis of these kinds of data.

Furthermore, alternative classifications for members of the class have been proposed. Moestrup (1982) resurrected the Loxophyceae *sensu* Christensen (1962), which included taxa with dorsiventral cells and flagellar hair-points (slender flagellar tips). In addition, Ettl (1966) grouped flagellates sharing certain features of cell symmetry into the Pedinomonadineae.

Despite these problems in classification and its pivotal position in the evolution of the green algae, the Micromonadophyceae is a relatively unstudied group of organisms (Norris 1980, Moestrup 1982, Melkonian 1984). Independent investigations often focus on different aspects of the cellular ultrastructure, making comparisons among taxa difficult. Moreover, interpretations of the morphological and ultrastructural data are sometimes conflicting (cf. Moestrup and Ettl 1979 and Rogers et al. 1981; Moestrup 1982 and Melkonian 1984; O'Kelly and Floyd 1984b and Melkonian 1984).

Our nuclear-encoded rRNA sequence project was designed to provide an independent data set to test phylogenetic hypotheses based on ultrastructural and morphological data. Since rRNA is found in all organisms and is evolutionarily conserved, its use permits comparisons of highly divergent taxa (Gunderson et al. 1987). In view of the presumed antiquity of the green algal lineages, conflicting hypotheses of green algal evolution, and disagreements over the relative importance of certain ultrastructural characters, the use of rRNA in a phylogenetic analysis of the green algae seems particularly appropriate.

MATERIALS AND METHODS

Twenty-two taxa representing the five classes of green algae, land plants, a diatom, and yeast were studied (Table 1). Cultures of marine organisms were maintained in *K* or *f/2* medium (Keller et al. 1987). Freshwater species were maintained in minimal medium (Starr 1978) or minimal medium supplemented with soil water. Batch cultures were grown in liter quantities and were bubbled with filtered air. All cultures were grown under a 16:8 h LD cycle. Cells were harvested by centrifugation and washed with 50 mM Tris HCl (pH 9.0).

RNA templates. To extract RNA, cells were lysed in a variety of ways. The most effective method for the micromonadophycean taxa was lysis in extraction buffer containing a 10% solution of sodium dodecylsulfate. A Paar bomb pressurized to 1500 psi was the most effective method for pleurastrophycean taxa. Purified high molecular weight RNA was isolated by a modification of the methods of Coleman and Grossman (1984). All RNA samples were concentrated to 1 mg·mL^{-1} and stored in 1 mM Tris-EDTA (pH 7.5) buffer at −20° C. Integrity of the rRNA was checked on agarose minigels.

Direct sequencing. Protocols for direct sequencing with reverse transcriptase, oligonucleotide primers, and deoxy- and dideoxynucleotides are a modification of Youvan and Hearst (1981) and Qu et al. (1983) by Hamby et al. (1988). Partial sequences of 150–200 nucleotides in length were generated from primers that are complementary to highly conserved regions of the 18S and 26S rRNA molecules. Six primers (18E, 18H, 18J, 18L, 26C, and 26D, see Hamby et al. 1988) yield partial sequences totalling approximately 1000–1200 nucleotides per species, representing 50% and 10% of the 18S and 26S rRNAs, respectively. Sequenced regions correspond to universal, semi-conserved, and variable regions (Gray et al. 1984), and have been used successfully in studies of land plant phylogeny (Hamby and Zimmer 1988).

Sequence alignment. The sequences were roughly aligned using the GAP, LINEUP, and PRETTY programs in the University of Wisconsin Genetics Users Group software package (UWGCG version 6.1, Devereux et al. 1984). Final alignments were done by hand. Regions of the sequences that were difficult to align with any confidence were excluded from the phylogenetic analysis, resulting in a final alignment of 890 base positions per taxon (Fig. 1). A total of 628 base positions were invariant or autapomorphic (and thus, uninformative) while 262 base positions (or about 29%) were informative and used in the phylogenetic analysis.

Phylogenetic analysis. Molecular data were analyzed using the tree-bisection-reconnection branch swapping algorithm in PAUP version 3.0g (Phylogenetic Analysis Using Parsimony, Swofford 1989), which is a heuristic method and does not guarantee to find the most parsimonious cladogram. To increase the probability of finding all most parsimonious trees the analysis was performed ten times with the taxa entered in random order. Trees were rooted using *Pharodactylum* and *Saccharomyces* (Rubstov et al. 1980) as outgroups. Bootstrap analysis (Felsenstein 1985) was

performed with PAUP version 3.0g (Swofford 1989). Non-molecular data were analyzed using the Branch and Bound algorithm in PAUP 3.0g (Swofford 1989). All characters were considered to be multistate and unordered.

RESULTS AND DISCUSSION

An analysis of the sequence data alone (262 informative characters) yielded three equally most parsimonious cladograms, each with a length of 861 steps and with a consistency index of 0.559 (Fig. 2A–C). The three cladograms differ only in the relative positions of *Pleurastrum* and *Bryopsis*.

Cladograms generated from the sequence data are closely congruent with that from the morphological and ultrastructural data (Mishler and Churchill 1985). In both analyses the Charophyceae represents a basal divergence relative to the other green algal classes. Furthermore, as in Mishler and Churchill (1985), the monophyly of the Ulvophyceae is not resolved in this analysis.

In two of the trees the Pleurastrophyceae (plus *Pyramimonas*) occupy a single clade (Fig. 2B, C). In the third tree *Pleurastrum* is basal to the other pleurastrophytes and to the Chlorophyceae (Fig. 2A). Thus, the sequence data alone do not resolve the issue of the monophyly of the Pleurastrophyceae. However, the pleurastrophycean taxa are on the same lineage as the Chlorophyceae but not that of the Ulvophyceae, which supports the classification of Mattox and Stewart (1984), but not that of Sluiman (1989).

Within the Pleurastrophyceae, several phylogenetic issues can be addressed. The sarcinoid genus *Friedmannia* was placed in the Ulotrichales of the Ulvophyceae by Lokhorst (1984) based, in part, on life history characters and features of flagellar ultrastructure. The sequence data indicate a closer affinity of *Friedmannia* with other pleurastrophycean taxa than with any members of the Ulvophyceae. In addition, *Tetraselmis* is placed in the Pleurastrophyceae by Mattox and Stewart (1984) (and on the "*Pleurastrum* lineage" by O'Kelly and Floyd [1984b]) based on details of mitosis (Stewart et al. 1974, Molnar et al. 1975) and flagellar apparatus ultrastructure (O'Kelly and Floyd 1984b). However, based on flagellar apparatus ultrastructure and flagellar scales Moestrup (1982) and Melkonian (1984) placed *Tetraselmis* in the Micromonadophyceae. The rRNA sequence data show a close relationship between *Tetraselmis* and the Pleurastrophyceae.

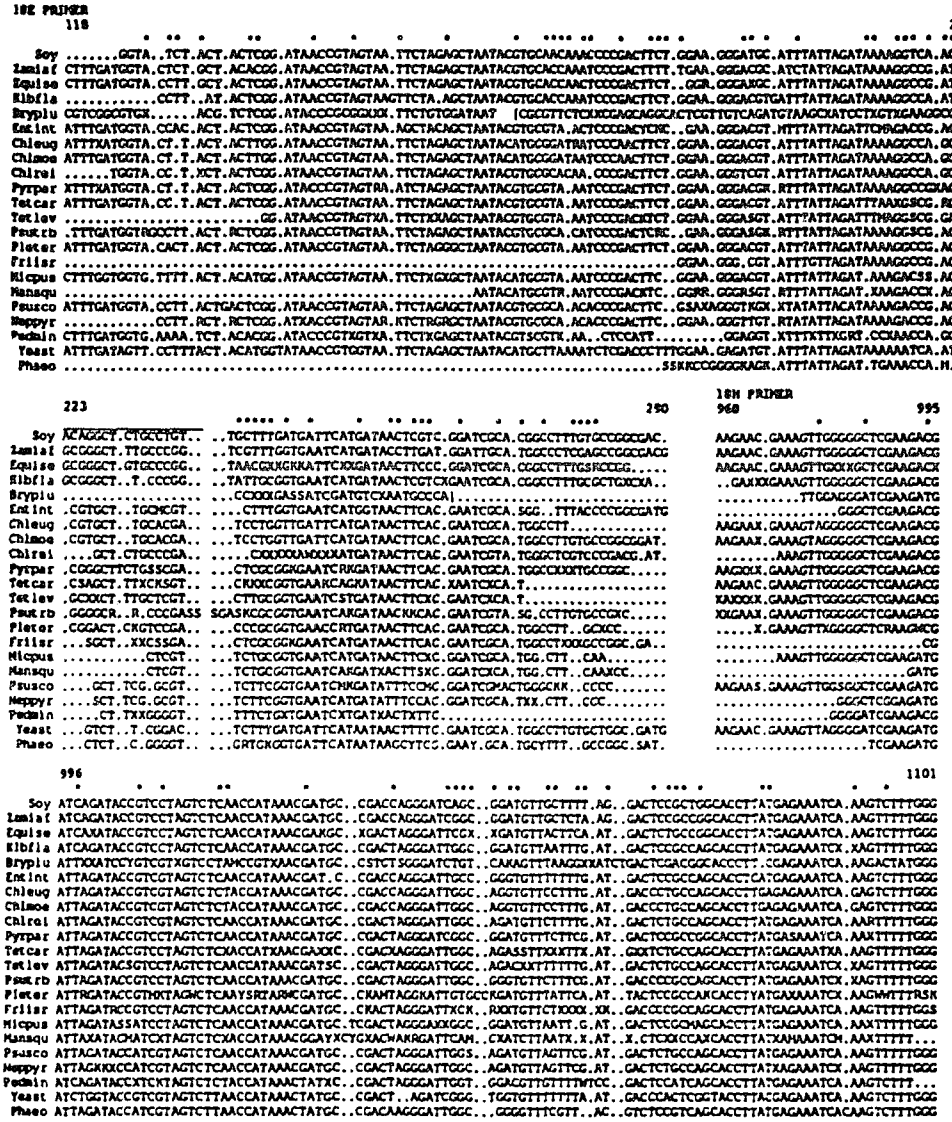
An interesting result of the analysis of the sequence data is the position of the scaled flagellate *Pyramimonas* on a clade with the Pleurastrophyceae. *Pyramimonas* is often considered a primitive micromonadophyte, possibly ancestral to the other classes of green algae (Norris 1980, O'Kelly and Floyd 1984b, van den Hoek et al. 1989). Its position on the pleurastrophyte lineage in our analysis suggests that it may not be a good example of a "primitive" flagellate. Its alliance with the pleurastrophytes may not be too surprising because of similarities of some aspects of the flagellar apparatus, flagellar scales, and cytokinesis in *Pyramimonas* and *Tetraselmis* (Pearson and Norris 1975, Norris 1980).

The sequence data indicate that the Micromonadophyceae is not monophyletic but is instead composed of at least three lineages. First, the position

TABLE 1. *List of organisms used in this study.*

	Source[a]
Micromonadophyceae	
Mantoniella squamata (Manton et Parke) Desikachary	CCMP PLY189
Micromonas pusilla (Butcher) Manton et Parke	CCMP DW8
Nephroselmis pyriformis (Butcher) Rayns	CCMP UW460
Pedinomonas minutissima Skuja	CCMP VA8
Pseudocourfieldia marina Throndsen	CCMP IVP11
Pyramimonas parkae Norris et Pierson	UTEX 2287
Tetraselmis carteriiformis Butcher	CCMP UW439
Tetraselmis levis Butcher	CCMP PLATY1
Pleurastrophyceae	
Friedmannia israelensis Chantanachat et Bold	UTEX 1181
Pleurastrum terrestre Fritsch et John	UTEX 555
Pseudotrebouxia gigantea Hildreth et Ahmadj.	UTEX 2251
Ulvophyceae	
Bryopsis plumosa[b] (Hudson) C. Ag.	
Enteromorpha intestinalis (L.) Link	UTEX LB2272
Chlorophyceae	
Chlamydomonas eugametos[c] Moewus	UTEX 9
Chlamydomonas moewusii[c] Gerloff	UTEX 97
Chlamydomonas reinhardtii[c] Dangeard	CC 124
Charophyceae	
Klebsormidium flaccidum[d] (Kutzing) Silver, Mattox et Blackwell	UTEX 321
Land plants	
Equisetum hyamale[e] L.	
Glycine max[f] (L.) Merr.	
Zamia floridana[g] L.	
Bacillariophyceae	
Phaeodactylum tricornutum[h] Bohlin	
Ascomycetes	
Saccharomyces cerevisiae[i] Hansen	

[a] CCMP = Center for the Culture of Marine Phytoflagellates, Bigelow Laboratory; UTEX = The Culture Collection of Algae at the University of Texas at Austin; CC = Collection of *Chlamydomonas* species at Duke University.
[b] Published sequence from Zechman et al. (1990).
[c] Published sequence from Buchheim et al. (1990).
[d] Unpublished sequence from D. A. Waters.
[e] Unpublished sequence from R. K. Hamby.
[f] Published sequence from Eckenrode et al. (1985).
[g] Unpublished sequence from M. Arnold.
[h] Material obtained from A. R. Grossman, Carnegie Institute, CA.
[i] Published sequence from Rubstov et al. (1980).


18E PRIMER
118
222
Soy .....GGTA..TCT.ACT.ACTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCAACAAACCCCGACTTCT.GGAA.GGGATGC.ATTTATTAGATAAAAGGTCA.AC..
Lmlaf CTTTGATGGTA.CTCT.GCT.ACACGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCACCAAATCCCGACTTTT.TGAA.GGGACGC.ATCTATTAGATAAAAGGCCG.AT..
Equise CTTTGATGGTA.CCTT.GCT.ACTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCACCAACTCCCGACTTCT..GGR.GGGAGGC.ATTTATTAGATAAAAGGCCG.AT..
Elbfla .....CCTT..AT.ACTCGG.ATAACCGTAGTAAGTTCTA.AGCTAATACGTGCACCAAATCCCGACTTCT.GGAA.GGGACGTGATTTATTAGATAAAAGCCA.AT..
Bryplu CGTCGGCGTCN.....ACG.TCTCGG.ATACCCGCGGGXXX.TTCTGTGGATAAT [CGCGTTCTCXXCGAGCAGGCACTCGTTGTCAGATGTAAGCXATCCTXGTXGAAGGCGAA
Entint ATTTGATGGTA.CCAC.ACT.ACTCGG.ATAACCGTAGTAA.AGCTACAGCTAATACGTGCGTA.ACTCCCGACTCXC..GAA.GGGACGT.ATTTATTAGATTCXAGACCG.AC..
Chleug ATTTXATGGTA.CT.T.ACT.ACTTGG.ATAACCGTAGTAA.TTCTAGAGCTAATACATGCGGATAATCCCAACTTCT.GGAA.GGGACGT.ATTTATTAGATAAAAGCCA.GC..
Chlmoe ATTTGATGGTA.CT.T.ACT.ACTTGG.ATAACCGTAGTAA.TTCTAGAGCTAATACATGCGGATAATCCCAACTTCT.GGAA.GGGACGT.ATTTATTAGATAAAAGCCA.GC..
Chlrei .....TGGTA.CC.T.XCT.ACTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCGCACAA.CCCGACTTCT.GGAA.GGGTCGT.ATTTATTAGATAAAAGCCA.GC..
Pyrpar XTTTXATGGTA.CT.T.ACT.ACTCGG.ATACCCGTAGTAA.ATCTAGAGCTAATACGTGCGTA.AATCCCGACTTCT.GGAA.GGGACGR.RTTTATTAGATAAAAGCCGXAC..
Tetcar ATTTGATGGTA.CC.T.ACT.ACTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCGTA.AATCCCGACTTCT.GGAA.GGGACGT.ATTTATTAGATTTAAXGSCG.RG..
Tetlev .....GG.ATAACCGTAGTXA.TTCTXXAGCTAATACGTGCGTA.AATCCCGACXTCT.GGAA.GGGASGT.ATTTATTAGATTTRAGGSCG.GA..
Psuxrb .TTTGATGGTRGCCTT.ACT.RCTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCGCA.CATCCCGACTCRC..GAA.GGGASGR.RTTTATTAGATAAAAGSCG.AGCC
Pleter ATTTGATGGTA.CACT.ACT.ACTCGG.ATAACCGTAGTAA.TTCTAGGGCTAATACGTGCGTA.AATCCCGACTTCT.GGAA.GGGACGT.ATTTATTAGATAAAAGCCG.AC..
Frilsr .....GGAA.GGG.CGT.ATTTGTTAGATAAAAGCCG.AC..
Micpus CTTTGGTGGTG.TTTT.ACT.ACATGG.ATAACCGTAGTAA.TTCTXGXGCTAATACATGCGTA.AATCCCGACTTC..GGAA.GGGACGT.ATTTATTAGAT.AAAGAC5S.AC..
Mansqu .....AATACATGCGTR.AATCCCGACXTC..GGRR.GGGRSGT.RTTTATTAGAT.XAAGACCX.AC..
Psusco ATTTGATGGTA.CCTT.ACTGACTCGG.ATAACCGTAGTAA.TTCTAGAGCTAATACGTGCGCA.ACACCCGACTTC..GSAXAGGGTXGX.XTATATTACATAAAAGACCG.AC..
Meppyr .....CCTT.RCT.RCTCGG.ATXACCGTAGTAR.RTCTRGRGCTAATACGTGCGCA.ACACCCGACTTC..GGAA.GGGTTGT.RTATATTAGATAAAAGACCG.AC..
Pedmin CTTTGATGGTG.AAAA.TCT.ACACGG.ATACCCGTXGTXA.TTCTXGAGCTAATACGTSCGTX.AA..CTCCATT.....GGAGGT.XTTTXTTXGRT.CCXAACCA.GC..
Yeast ATTTGATAGTT.CCTTTACT.ACATGGTATAACCGTGGTAA.TTCTAGAGCTAATACATGCTTAAAATCTCGACCCTTTGGAA.GAGATGT.ATTTATTAGATAAAAATCA.AT..
Phaeo .....5SKRCCGGGGGXAGR.ATTTATTAGAT.TGAAACCA.M...
223
290
18N PRIMER
960
995
Soy XCAGGCT.CTGCCTGT.. ..TGCTTTGATGATTCATGATAACTCGTC.GGATCGCA.CGGCCTTTGTGCCGGCCAC. AAGAAC.GAAAGTTGGGGGCTCGAAGACG
Lmlaf GCGGGCT.TTGCCCGG.. ..TCGTTTGGTGAATCATGATACCTTGAT.GGATTGCA.TGGCCCTCGAGCCGGCCACG AAGAAC.GAAAGTTGGGGGCTCGAAGACG
Equise GCGGGCT.GTGCCCGG.. ..TAACGGNGKKATTCXXGATAACTTCCC.GGATCGCA.CGGCCTTTGSRCCGG..... AAGAAC.GAAAGTTGXXXGCTCGAAGACX
Elbfla GCGGGCT..T.CCCGG.. ..TATTGCGGTGAATCATGATAACTCGTCXGAATCGCA.CGGCCTTTGCGCTGXCXA.. ..GAXXXGAAAGTTGGGGGCTCGAAGACG
Bryplu ....CCXXXGASSATCGATGTCXAATGCCCA|..... .....TTGCAGGGATCGAAGATG
Entint .CGTGCT..TGCMCGT.. ....CTTTGGTGAATCATGGTAACTTCAC.GAATCGCA.SGG..TTTACCCCGGCGATG .....GGGCTCGAAGACG
Chleug .CGTGCT..TGCACGA.. ...TCCTGGTTGATTCATGATAACTTCAC.GAATCGCA.TGGCCTT..... AAGAAX.GAAAGTAGGGGGCTCGAAGACG
Chlmoe .CGTGCT..TGCACGA.. ...TCCTGGTTGATTCATGATAACTTCAC.GAATCGCA.TGGCCTTGTGCCGGCGGAT. AAGAAX.GAAAGTAGGGGGCTCGAAGACG
Chlrei ....GCT.CTGCCCGA.. .....CXXXXXAXXXXATGATAACTTCAC.GAATCGTA.TGGGCTCGTCCCGACG.AT. .....AAAGTTGGGGGCTCGAAGACG
Pyrpar .CGGGCTTCTGSSCGA.. ...CTCGCGGNGAATCRNGATAACTTCAC.GAATCGCA.TGGCCXXXTGCCGGC..... AAGCXX.GAAAGTTGGGGGCTCGAAGACG
Tetcar .CSAGCT.TTXCKSGT.. ...CKXXCGGTGAAKCAGXATAACTTCAC.XAATCXCA.T..... AAGAAC.GAAAGTTGGGGGCTCGAAGACG
Tetlev .GCXXCT.TTGCTCGT.. ...CTTGCGGTGAATCSTGATAACTTCXC.GAATCXCA.T..... XAXXXX.GAAAGTTGGGGGCTCGAAGACG
Psuxrb .GGGGCR..R.CCCGASS SGASKCGCGGTGAATCARGATAACKKCAC.GAATCGTA.SG.CCTTGTGCCGXC..... XXGAAX.GAAAGTTGGGGGCTCGAAGACG
Pleter .CGGACT.CKGTCCGA.. ...CCCGCGGTGAACCRTGATAACTTCAC.GAATCGCA.TGGCCTT..GCXCC..... .....X.GAAAGTTXGGGGCTCRAAGMCG
Frilsr ...SGCT..XXCSSGA.. ...CTCGCGGNGAATCATGATAACTTCAC.GAATCGCA.TGGCCTXXXGCCGGC.GA.. .....CG
Micpus .....CTCGT.. ...TCTGCGGTGAATCATGATAACTTCXC.GGATCGCA.TGG.CTT..CAA..... .....AAAGTTGGGGGCTCGAAGATG
Mansqu .....CTCGT.. ...TCTGCGGTGAATCAAGATXACTTSXC.GGATCXCA.TGG.CTT..CAAXCC..... .....GATG
Psusco ....GCT.TCG.GCGT.. ...TCTTCGGTGAATCHNGATATTTCCNC.GGATCGMACTGGGCNN..CCCC..... AAGAAS.GAAAGTTGGSGGCTCGAAGATG
Meppyr ....SCT.TCG.GCGT.. ...TCTTCGGTGAATCATGATATTTCCAC.GGATCGCA.TXX.CTT..CCC..... .....GGGCTCGGAGATG
Pedmin .....CT.TXXGGGGT.. ...TTTCTGXTGAATCXTGATXACTXTTC..... .....GGGGATCGAAGACG
Yeast ...GTCT..T.CGGAC.. ...TCTTYGATGATTCATAATAACTTTTTC.GAATCGCA.TGGCCTTGTGCTGGC.GATG AAGAAC.GAAAGTTAGGGGATCGAAGATG
Phaeo ...CTCT..C.GGGGT.. ...GRTGNGGTGATTCATAATAAGCYTCG.GAAY.GCA.TGCYTTT..GCCGGC.SAT. .....TCGAAGATG
996
1101
Soy ATCAGATACCGTCCTAGTCTCAACCATAAACGATGC..CGACCAGGGATCAGC..GGATGTTGCTTTT.AG..GACTCCGCTGGCACCTTATGAGAAATCA.AAGTCTTTGGG
Lmlaf ATCAGATACCGTCCTAGTCTCAACCATAAACGATGC..CGACCAGGGATCGGC..GGATGTTGCTCTA.AG..GACTCCGCCGGCACCTTATGAGAAATCA.AAGTTTTTGGG
Equise ATCAAATACCGTCCTAGTCTCAACCATAAACGAAGC..XGACTAGGGATTCGX..XGATGTTACTTCA.AT..GACTCTGCCGGCACCTTATGAGAAATCA.AAGTCTTTGGG
Elbfla ATCAGATACCGTCCTAGTCTCAACCATAAACGATGC..CGACTAGGGATTGGC..GGATGTTAATTTG.AT..GACTCCGCCAGCACCTTATGAGAAATCX.XAGTTTTTGGG
Bryplu ATTXXATCCYGTCGTXGTCCTAMCCGTXAACGATGC..CSTCTSGGGATCTGT..CAAAGTTTAAGGXXATCTGACTCGACGGCACCCTT.CGAGAAATCA.AAGACTATGGG
Entint ATTAGATACCGTCGTAGTCTCAACCATAAACGAT.C..CGACCAGGGATTGCC..GGGTGTTTTTTTG.AT..GACTCCGCCAGCACCTCA'GAGAAATCA.AAGTCTTTGGG
Chleug ATTAGATACCGTCGTAGTCTCTACCATAAACGATGC..CGACCAGGGATTGGC..AGGTGTTCCTTTG.AT..GACCCTGCCAGCACCTTGAGAGAAATCA.GAGTCTTTGGG
Chlmoe ATTAGATACCGTCGTAGTCTCTACCATAAACGATGC..CGACCAGGGATTGGC..AGGTGTTCCTTTG.AT..GACCCTGCCAGCACCTTGAGAGAAATCA.GAGTCTTTGGG
Chlrei ATTAGATACCGTCGTAGTCTCAACCATAAACGATGC..CGACTAGGGATTGGC..AGATGTTCTTTTG.AT..GACTCTGCCAGCACCTTATGAGAAATCA.AARTTTTTGGG
Pyrpar ATTAGATACCGTCCTAGTCTCAACCATAAACGATGC..CGACTAGGGATCGGC..GGATGTTTCTTCG.AT..GACTCCGCCGGCACCTTATGASAAATCA.AAXTTTTTGGG
Tetcar ATTAGATACCGTCCTAGTCTCXACCATXAACGAXXC..CGACXAGGGATTGGC..AGASSTTXXKTTX.AT..GXXTCTGCCAGCACCTTATGAGAAATXA.AAGTTTTTGGG
Tetlev ATTAGATACSGTCCTAGTCTCAACCATAAACGATSC..CGACTAGGGATTGGC..AGACXXTTTTTTG.AT..GACTCTGCCAGCACCTTATGAGAAATCX.XAGTTTTTGGG
Psuxrb ATTAGATACCGTCCTAGTCTCAACCATAAACGATGC..CGACTAGGGATTGGC..GGGTGTTCTTTCG.AT..GACCCCGCCAGCACCTTATGAGAAATCX.XAGTTTTTGGG
Pleter ATTRGATACCGTMXTAGMCTCAAYSRTARMCGATGC..CKAMTAGGKATTGTGCCRGATGTTTATTCA.AT..TACTCCGCCAXCACCTYATGAXAAATCX.AAGWWTTTRSK
Frilsr ATTAGATRCCGTCCTAGTCTCAACCATAAACGATGC..CKACTAGGGATTXCK..RXXTGTTCTXXXX.XK..GACCCCGCCAGCACCTTATGAGAAATCA.AAGTTTTTGGS
Micpus ATTAGATASSATCCTAGTCTCAACCATAAACGATGC.TCGACTAGGGAXXCGGC..GGATGTTAATT.G.AT..GACTCCGCHAGCACCTTATGAGAAATCA.AAXTTTTTGGG
Mansqu ATTAXATACMATCXTAGTCTCXACCATAAACGGAYXCYGXACWAMRGATTCAM..CXATCTTAATX.X.AT..X.CTCXXCCXXCACCTTATXAHAAATCN.AAXTTTTT...
Psusco ATTAGATACCATCGTAGTCTCAACCATAAACGATGC..CGACTAGGGATTGGS..AGATGTTAGTTCG.AT..GACTCTGCCAGCACCTTATGAGAAATCA.AAGTTTTTGGG
Meppyr ATTAGKKXCCATCGTAGTCTCAACCATAAACGATGC..CGACTAGGGATTGGC..AGATGTTAGTTCG.AT..GACTCTGCCAGCACCTTATXAGAAATCX.AAGTTTTTGGG
Pedmin ATCAGATACCXTCKTAGTCTCTACCATAAACTATXC..CGACTAGGGATTGGT..GGACGTTGTTTTWTCC..GACTCCATCAGCACCTTATGAGAAATCA.AAGTCTTT...
Yeast ATCTGGTACCGTCGTAGTCTTAACCATAAACTATGC..CGACT..AGATCGGG..TGGTGTTTTTTTA.AT..GACCCACTCGGTACCTTACGAGAAATCA.AAGTCTTTGGG
Phaeo ATTAGATACCATCGTAGTCTTAACCATAAACTATGC..CGACAAGGGATTGGC...GGGGTTTCGTT..AC..GTCTCCGTCAGCACCTTATGAGAAATCACAAGTCTTTGGG

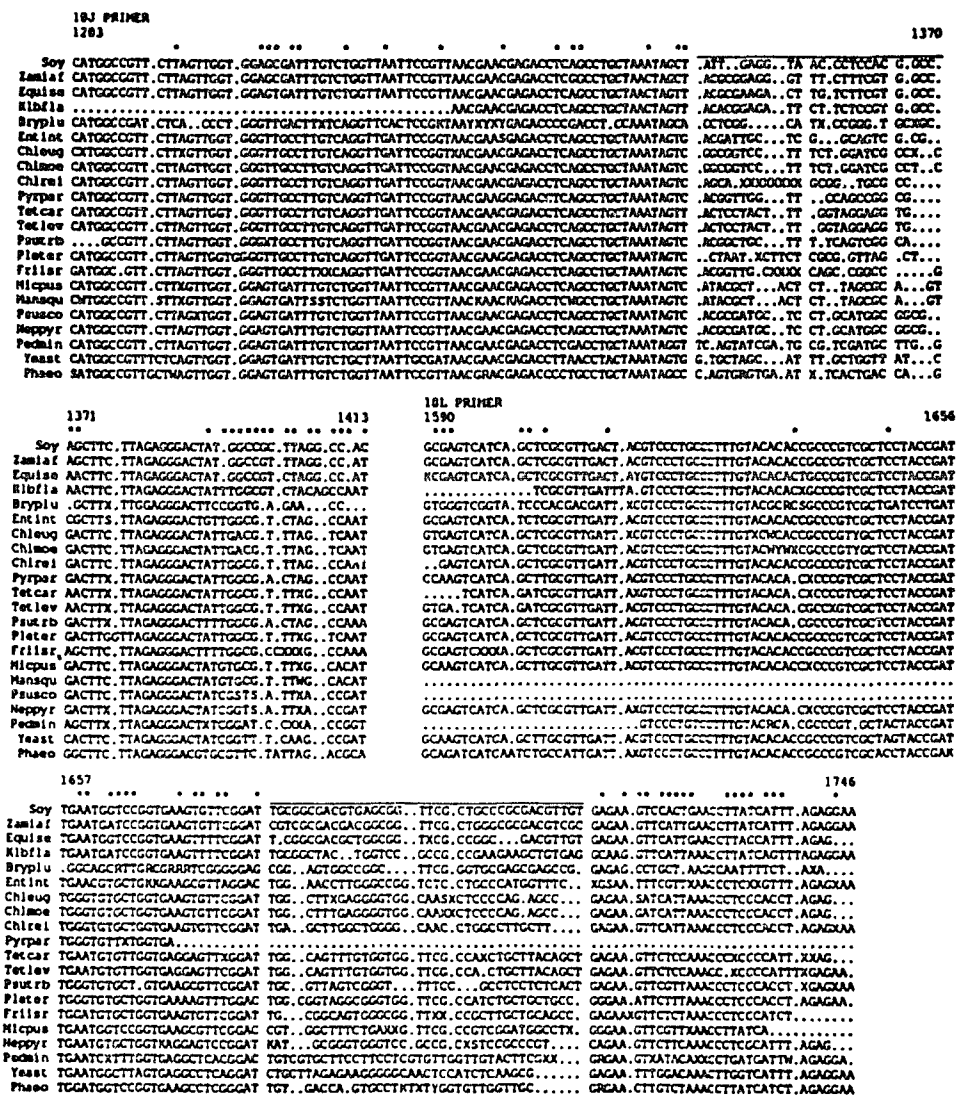

18J PRIMER
18L PRIMER

FIG. 1. Aligned sequences for each primer and each taxon included in the phylogenetic analysis. Phylogenetically informative sites are denoted by an asterisk above the base position. Highly variable regions which were excluded from the analysis are denoted by a solid line above the alignment. Ambiguous sites were coded using the nomenclature proposed by IUB (1985). Gaps are denoted by periods. Abbreviations for the taxa are as follows: Soy = *Glycine max*, Zamiaf = *Zamia floridana*, Equise = *Equisetum hyamale*, Klbfla = *Klebsormidium flaccidum*, Bryplu = *Bryopsis plumosa*, Entint = *Enteromorpha intestinalis*, Chleug = *Chlamydomonas eugamtos*, Chlmoe = *C. moewusii*, Chlrei = *C. reinhardtii*, Pyrpar = *Pyramimonas parkae*, Tetcar = *Tetraselmis carteriiformis*, Tetlev = *T. levis*, Psutrb = *Pseudotrebouxia gigantea*, Pleter = *Pleurastrum terrestre*, Friisr = *Friedmannia israelensis*, Micpus = *Micromonas pusilla*, Mansqu = *Mantoniella squamata*, Psusco = *Pseudocourfieldia marina*, Neppyr = *Nephroselmis pyriformis*, Pedmin = *Pedinomonas minutissima*, Yeast = *Saccharomyces cerevisear*, and Phaeo = *Pharodactylum triconutum*.

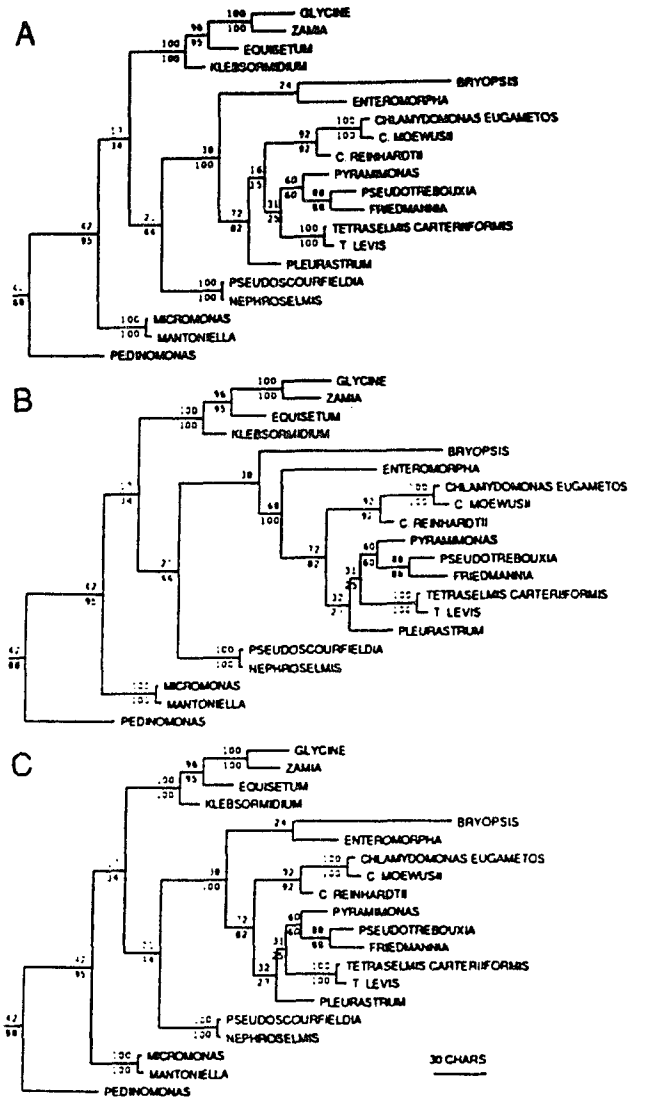


FIG. 2A–C. Three equally parsimonious cladograms generated from the rRNA sequence data alone. Each cladogram has a length of 861 steps and a consistency index of 0.559. The *f*-values for A, B, and C are 2260, 5512, and 2490, respectively. Numbers above the branches are the bootstrap values when all of the taxa were included in the analysis. Numbers below the branches are the bootstrap values when *Bryopsis* was excluded from the analysis. Branch lengths are proportional to the number of characters supporting each clade (a scale is provided).

of *Pedinomonas*, basal to all the other green algae, is a consistent feature of the rRNA cladograms. *Pedinomonas* has a simple cellular organization, with one flagellum, one nucleus, one chloroplast, one mitochondrion, one golgi apparatus, and no cellular covering (Ettl and Manton 1964, Ettl 1972), and features of its cell division are considered to be primitive for the green algae (Pickett-Heaps and Ott

TABLE 2. *Twelve non-molecular characters for the taxa examined in this study. Characters were collected from recent reviews of green algal systematics and are listed in Table 3.*

1	2	3	4	5	6	7	8	9	10	11	12	Taxa
0	0	0	1	0	1	0	0	0	1	1	0	Charophycean taxa
1	1	0	0	0	0	0	0	0	0	0	0	Ulvophycean taxa
1	1	1	0	2	0	1	0	0	0	0	0	Chlorophycean taxa
1	1	0	0	0	0	1	1	1	0	0	0	Pleurastrophycen taxa
0	0	0	0	1	0	0	0	0	0	0	1	*Mantoniella*
0	0	0	0	0	0	0	0	0	0	0	1	*Micromonas*
0	0	0	0	1	0	0	0	0	0	0	0	*Nephroselmis*
0	0	0	0	0	0	0	0	0	0	0	0	*Pedinomonas*
0	0	0	0	1	0	0	0	0	0	0	0	*Pseudoscourfieldia*
0	0	0	0	1	0	0	0	0	0	0	0	*Pyramimonas*
0	0	0	0	1	0	1	1	0	0	0	0	*Tetraselmis*

1974). Its basal position within the cladogram raises the possibility that its simple structure may be due to a retention of primitive features rather than an evolutionary reduction. Second, the close relationship between the scaled *Mantoniella* and the scaleless *Micromonas* in the rRNA cladograms corroborates studies of their chloroplast biochemistry (Wilhelm et al. 1986, Fawley et al. 1986). Finally, the clade formed by *Nephroselmis* and *Pseudoscourfieldia* is consistent with the flagellar transition region data (Melkonian 1984).

User-defined trees. Alternative hypotheses of green algal evolution were tested by comparing the lengths of user-defined trees and lengths of the most parsimonious trees. Under the principle of parsimony, trees representing alternative hypotheses of evolution show increasingly less support from the data as the lengths of the trees increase.

Rearrangements of taxa within the Pleurastrophyceae (plus *Pyramimonas*) clade generated trees no more than five steps longer than the most parsimonious tree. Moving *Pyramimonas* to a position basal to a monophyletic Pleurastrophyceae resulted in a tree five steps longer than the most parsimonious. Moving *Pyramimonas* and *Tetraselmis* to the base of the Chlorophyceae, thereby creating a clade of flagellates, produced a tree 14 steps longer than the most parsimonious. As a test of the Sluiman (1989) classification, pleurastrophyte taxa (not including *Pyramimonas*) were moved to the base of a monophyletic Ulvophyceae, which generated a tree 19 steps longer than the most parsimonious tree.

Rearrangements of taxa within the pleurastrophycean clade do not increase the length of the tree by more than a few steps, and the addition of more sequence data and more taxa in future studies may alter the relationships within the group. However, moving members of the class either to the Chlorophyceae or the Ulvophyceae increased the length of the trees substantially, which indicates that these hypotheses of evolution have far less support from the data.

Making the *Pseudoscourfieldia/Nephroselmis* clade and the *Micromonas/Mantoniella* clade either a single clade or a grade basal to the rest of the green algal classes produces trees of two to four steps longer than the most parsimonious. However, a test of the Loxophyceae *sensu* Christensen (Moestrup 1982) and of the Pedinomonadineae (Ettl 1966), i.e. moving *Micromonas* to the *Pedinomonas* lineage, resulted in a tree 29 steps longer. Clearly, the rRNA sequence data show little support for either the concept of the Loxophyceae or the Pedinomonadineae.

Bootstrap analysis. Bootstrapping is a statistical resampling procedure, in which the characters within a data set are sampled randomly, with replacement, to construct a new data set of the same size as the original. Tree construction is then performed with the new data set. The bootstrap is replicated many times (at least 20 replicates is recommended [Felsenstein 1989]) and the frequency of occurrence of each monophyletic group of taxa is recorded (Felsenstein 1985). Since the data are sampled at random, those monophyletic groups with many supporting characters will tend to appear more frequently in the bootstrap analyses than those with little support. Thus, bootstrapping is often used as a method for assigning "confidence limits" to the nodes of a cladogram (Felsenstein 1985, Sanderson 1989).

Two bootstrap analyses were performed, each with one hundred replications. Bootstrap frequencies are indicated on the most parsimonious trees (Fig. 2A–C). The first bootstrap analysis included all the taxa. High values (greater than 80%) were found in the Charophyceae/land plant clade and the Chlorophyceae clade. In addition, clades of *Micromonas* and *Mantoniella*, *Nephroselmis* and *Pseudoscourfieldia*, *Friedmannia* and *Pseudotrebouxia*, and the two *Tetraselmis* species had high bootstrap frequencies. Low values (less than 80%) were observed at nodes among the pleuroastrophyte taxa, at the node below the chloro-/pleurastrophyte clade, at the node below the ulvo-/chloro-/pleurastrophyte clade, and at the nodes at the base of the tree where the charophyte/land plant and the several micromonad lineages diverge.

The bootstrap frequencies indicate that certain

TABLE 3. *Twelve ultrastructural and biochemical characters collected from recent reviews of green algal systematics. Symbols for the character states are in parentheses.*

1. Cruciate flagellar root system (0 = absent; 1 = present)
2. Striated microtubule associated complex (0 = absent; 1 = present)
3. Clockwise basal body orientation (0 = absent; 1 = present)
4. Multilayered structure (0 = absent; 1 = present)
5. Motile cell covering (0 = naked; 1 = scales or fused scales; 2 = cell wall)
6. Phragmoplast/cell plate (0 = absent; 1 = present)
7. Collapsing spindle (0 = absent; 1 = present)
8. Metacentric mitotic spindle apparatus (0 = absent; 1 = present)
9. Microtubules cup nucleus during mitosis (0 = absent; 1 = present)
10. Open spindles (0 = absent; 1 = present)
11. Glycollate oxidase (0 = absent; 1 = present)
12. Size of the light-harvesting pigment-protein complex (0 = small; 1 = large)

clades not found in the most parsimonious cladograms were nonetheless common in the bootstrap analysis. In particular, *Bryopsis* appeared as the sister taxon to the outgroup taxon *Phaeodactylum* in 51% of the bootstrap replicates and appeared in several other clades at lower frequency. Because the position of *Bryopsis* was unstable relative to the other taxa, a second bootstrap analysis was performed excluding *Bryopsis*. In the second bootstrap analysis the frequencies of several clades increased to values above 80%. The chloro-/pleurastrophyte clade was found in 82% of the bootstrap replicates; the ulvo-/chloro-/pleurastrophyte clade was found in 100% of the bootstrap replicates; and the clade containing all the green algae except *Pedinomonas* was found in 95% of the bootstrap replicates.

These results underscore the fact that great care must be taken when interpreting the results of a bootstrap analysis (Sanderson 1989). If bootstrapping is to be performed at all, the limitations to the method must be recognized. Bootstrap frequencies provide no information about the relationships of the taxa above the node, nor do they provide information about the relationship of a clade to the rest of the tree (Sanderson 1989). Furthermore, low bootstrap frequencies for a clade may not be due solely to a lack of character support, but may have other causes. As was the case with *Bryopsis*, if the position of one taxon is uncertain, even though the relationships among the rest of the taxa are well supported, then the bootstrap frequencies of other clades on the tree may be lowered considerably.

Non-molecular data A preliminary data set of twelve ultrastructural and biochemical characters (Tables 2, 3) was constructed in order to compare the results of the molecular sequence data with an independent data set. The characters were from recent papers on green algal evolution (Mattox and Stewart 1984, Melkonian 1984, O'Kelly and Floyd 1984a, b, Mishler and Churchill 1985, Sluiman 1985, Fawley et al. 1986, Theriot 1988). The cladistic analysis resulted in 24 most parsimonious trees, none fully resolved, representing five topologies (cladograms not shown). The poor resolution of the trees was due primarily to the lack of characters supporting relationships within the Micromonadophyceae. The strict consensus tree (Fig. 3) shows the Pleurastrophyceae as sister group to the Chlorophyceae, and the Ulvophyceae sister group to the Chlorophyceae/Pleurastrophyceae clade. These relationships are closely congruent with the cladistic analysis of Mishler and Churchill (1985) and with the rRNA sequence data. The strict consensus tree also shows the Charophyceae, the Ulvo-/Chloro-/Pleurastrophyceae clade, *Tetraselmis*, and the micromonadophycean taxa emerging from an unresolved node. These relationships are also congruent with those of Mishler and Churchill (1985), though the sequence data showed resolution for these taxa.

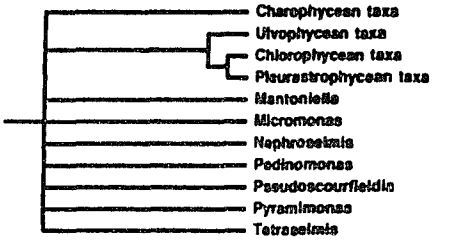


FIG. 3. Strict consensus tree of five most parsimonious topologies resulting from cladistic analysis of twelve non-molecular characters (Tables 2, 3).

A cladistic analysis of the combined molecular and non-molecular data, with all characters weighted equally, yielded two equally parsimonious trees, of identical topology to two of the cladograms obtained using sequence data alone (Fig. 2B, C). In cladograms from the combined data sets, the monophyly of the Ulvophyceae is unresolved, just as it is in the analysis of sequence data alone. However, the monophyly of the Pleurastrophyceae (including *Pyramimonas*) is resolved in the analysis of the combined data. Thus, the combination of the morphology and the sequence data provided greater resolution of the relationships among green algal taxa than did either data set alone.

CONCLUSIONS

Cladistic analysis of nuclear-encoded rRNA sequence data is a powerful method for reconstructing green algal phylogeny. From our analysis we draw several conclusions: 1) The Pleurastrophyceae is the sister group to the Chlorophyceae and not to the Ulvophyceae. 2) The Micromonadophyceae is not monophyletic. 3) *Pyramimonas*, traditionally considered a micromonad, is most closely allied with pleu-

rastrophycean taxa. 4) *Pedinomonas minutissima* is the earliest diverging green alga examined in this study. 5) Neither the Loxophyceae *sensu* Christensen (Moestrup 1982) nor the Pedinomonadineae (Ettl 1966) is supported by analysis of the rRNA sequence data.

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CHAPTER THREE

THE PHYLOGENY OF THE PRASINOPHYCEAE AND THE PLEURASTROPHYCEAE INFERRED FROM rRNA AND rDNA SEQUENCE DATA

INTRODUCTION

Evolutionarily conserved regions of the small subunit (SSU) and large subunit (LSU) ribosomal RNAs from five classes of green algae were sequenced and analyzed phylogenetically (Kantz et al. 1990) to test alternative hypotheses of green algal phylogeny (Mattox and Stewart 1984, O'Kelly and Floyd 1984, van den Hoek 1988, Moestrup and Throndsen 1988, Melkonian 1990). The study focused on the two problematic classes, Prasinophyceae (= Micromonadophyceae) and Pleurastrophyceae, and the analysis resulted in three equally parsimonious cladograms. The Prasinophyceae was not monophyletic in any of the cladograms, and the monophyly of the Pleurastrophyceae was ambiguous.

Ultrastructural and biochemical characters were also included in the study. The non-molecular data set was quite limited, including only 12 characters, and it showed little resolution among the taxa in the study. Nonetheless, the analysis of the combined independent data sets resolved relationships that

were ambiguous in each data set alone, because relationships that were poorly supported in one data set were better supported in the other data set. In addition to providing greater resolution, the combination of independent data sets has been defended on philosophical grounds. Miyamoto (1985) and Kluge (1989) have argued that the most parsimonious cladograms from combined data sets have the greatest explanatory power for all the characters available, and are therefore to be preferred.

This study includes previously published rRNA sequences (Kantz et al. 1990) and unpublished SSU and LSU rDNA sequences from additional members of the Pleurastrophyceae and the Prasinophyceae. The rDNA sequences were generated from Polymerase Chain Reaction (PCR) amplified DNA fragments (White et al. 1990, Kaltenboeck 1992). In addition, this study assesses the ratio of transitional and transversional changes and the degree of randomness in the data to test whether the use of ribosomal sequence data is appropriate for the phylogenetic level examined. A combined data set of the sequence data and 44 non-molecular characters (see chapter one) is analyzed

cladistically to test alternative hypotheses regarding the characteristics of the hypothetical ancestral flagellate.

MATERIALS AND METHODS

Thirty-four taxa representing the five classes of green algae, land plants, a diatom, a brown alga, and yeast were studied (Table 3.1). Because Polymerase Chain Reaction amplification methods were used, only small amounts of starting tissue were necessary, so that the difficulties of batch culturing were avoided. Indeed, many prasinophycean taxa are difficult to maintain in culture (Ricketts 1974, Moestrup 1991), possibly due to unusual nutrient requirements (Ricketts 1974). A sudden loss of batch cultures occurs frequently, and may be caused by virus infections (Melkonian 1982, 1990).

Table 3.1. List of organisms used in this study. CCMP=Provasoli-Guillard Center for Culture of Marine Phytoflagellates, Bigelow Laboratory UTEX=The Culture Collection of Algae at the University of Texas at Austin CC=Collection of Chlamydomonas species at Duke University. SAG=Sammlung von Algenkulturen, Göttingen.

Species by group

Micromonadophyceae
- Mantoniella squamata (Manton et Parke) Desikachary [CCMP PLY189]
- Mesostigma viride Lauterborn [SAG 50-1]
- Micromonas pusilla (Butcher) Manton et Parke [CCMP DW8]
- Nephroselmis pyriformis (Butcher) Rayns [CCMP UW460]
- Pedinomonas minor Korschikoff [SAG 1965-3]
- Pedinomonas minutissima Skuja [CCMP VA3]
- Pedinomonas tuberculata Vischer [SAG 42.84]
- Pseudoscourfieldia marina Throndsen [CCMP IVP11]
- Pyramimonas parkae Norris et Pierson [UTEX 2287]
- Pyramimonas virginica Norris et Pierson
- Tetraselmis carteriiformis Butcher [CCMP UW439]
- Tetraselmis levis Butcher [CCMP PLATY1]

Pleurastrophyceae
- Chlorosarcina longispinosa
- Friedmannia israelensis Chantanachat et Bold [UTEX 1181]
- Microthamnion kutzingianum
- Myrmecia biatorellae
- Pleurastrum terrestre Fritsch et John [UTEX 333]
- Pseudtrebouxia gigantea Hildreth et Ahmadj. [UTEX 2231]

Ulvophyceae
- Cladophora albida[a] (Hudson) Kütz
- Bryopsis plumosa[a] (Hudson) C. Ag.
- Enteromorpha intestinalis (L.) Link [UTEX LB2272]

Chlorophyceae
- Asteromonas gracilis Artari
- Chlamydomonas eugametos[b] Moewus [UTEX 9]
- Chlamydomonas moewusii[b] Gerloff [UTEX 97]
- Chlamydomonas reinhardtii[b] Dangeard [CC 124]
- Chlorella vulgaris Beij.
- Nanochlorum sp.

Charophyceae
- Klebsormidium flaccidum[c] (Kutzing) Silver, Mattox et Blackwell [UTEX 321]

Land Plants
- Equisetum hyamale[d] L.
- Glycine max[e] (L.) Merr.
- Zamia floridana[f] L.

Bacillariophyceae
- Phaeodactylum triconutum[g] Bohlin

Ascomycetes
- Saccharomyces cereviseae[h] Hansen

Phaeophyta
- Costaria costata[i]

[a] Sequence from F. W. Zechman [b] Sequence from M. A. Buchheim [c] Sequence from D. A. Waters [d] Sequence from R. K. Hamby [e] Published sequence from Eckenrode et al. (1985) [f] Sequence from M. Arnold [g] Material obtained from A. R. Grossman, Carnegie Institute, CA [h] Published sequence from Rubstov et al. (1980) [i] Published sequence from Battacharya and Druehl (1988)

Culturing. Cultures of marine organisms were maintained in 5 ml aliquots of K or f/2 medium (Keller et al. 1987). Freshwater species were maintained in minimal medium (Starr 1978) or minimal medium supplemented with soil water. All cultures were grown under a 16:8 h LD cycle.

DNA templates. The method of nucleic acid extraction is described in detail in Chapter Two and Chapter Four. Prasinophycean cells were lysed in extraction buffer containing a 1-2% solution of sodium dodecylsulfate. Pleurastrophycean cells were lysed in extraction buffer by sonication with short pulses for one minute. All DNA samples were concentrated to 1 $mg \cdot mL^{-1}$ and stored in 1 mM Tris-EDTA (pH 7.5) buffer at -20° C. Integrity of the DNA was checked on agarose minigels.

PCR Protocol. The production of PCR-amplified linear DNA templates followed the method of Koeltenboek et al. (1990). The method produces double-stranded DNA in a symmetric amplification step. The double-stranded product is used as a template in an asymmetric amplification step to produce single-stranded DNA for direct dideoxy

sequencing. Primers used in the PCR reactions were NS1 (White et al. 1990), 18H, 18L, 26B, and 26F (Hamby et al. 1988). The small subunit rDNA was amplified in two pieces. The primers NS1 and 18H amplified a region approximately 1100 bp in length. The primers 18L and the reverse complement of 18H amplified a region approximately 550 bp in length. A fragment of the large subunit rDNA approximately 1100 bp in length was amplified using the primers 26F and the reverse complement of 26B.

The symmetric amplification step used a reaction mixture of 10-200 ng complex DNA, 20-50 pmoles of both primers, 50-100 µmoles of dNTPs, and 2.0 units Taq Polymerase. The optimum temperature cycle for the symmetric amplification step was found to be: 93° C for 3 minutes, 51° C for 1 minute, 72° C for 1.5 minutes (for 1 cycle); 93° C for 1 minute, 51° C for 1 minute, 72° C for 1.5 minutes (for 25-29 cycles); 72° C for 5 minutes; 4° C soak.

The asymmetric amplification step used a reaction mixture of 10 µl symmetric PCR product, 20-50 pmoles of one primer (NS1, reverse complement of 18H, or reverse complement of 26B), 50-100 µmoles of

dNTPs, and 2.0 units Taq Polymerase. The optimum temperature cycle for the asymmetric amplification step was found to be: 93° C for 3 minutes, 53° C for 1 minute, 72° C for 1.5 minutes (for 1 cycle); 93° C for 1 minute, 53° C for 1 minute, 72° C for 1.5 minutes (for 19 cycles); 72° C for 5 minutes; 4° C soak.

Sequencing Protocol

The single-stranded asymmetric PCR product was separated from unincorporated primers, dNTPs, and salts by centrifugal ultracentrifugation (described in chapter 4). Sequencing of the asymmetric PCR product followed the protocols provided with the Sequenase sequencing kit (USB). Best results were obtained using 5-10 pmol of sequencing primer per reaction, and when the dGTP labeling solution was diluted 15-20 fold. ^{35}S-dATP-labeled reaction products were separated by 6% polyacrylamide-urea gel electrophoresis and visualized by autoradiography. Partial sequences of 200-300 nucleotides in length were generated from each of seven primers (18E, 18G, 18H, 18J, 18L, 26C, and 26D, see Hamby et al. 1988)

that are complementary to highly conserved regions of the small subunit (18S) and large subunit (26S) rRNA molecules.

Sequence alignment. The sequences were aligned first using the GAP, LINEUP, and PRETTY programs in what was the University of Wisconsin Genetics Users Group software package (UWGCG version 6.1, Devereux 1984) and is now the GCG software package. Final alignments were performed by hand. Regions of the sequences that were difficult to align were excluded from the phylogenetic analysis, resulting in a final alignment of 1576 base positions per taxon (Appendix A). A total of 1167 base positions were invariant or autapomorphic (and thus, uninformative), and 409 base positions (or about 35%) were informative and used in the phylogenetic analysis.

Phylogenetic analysis. Molecular data were analyzed using the tree-bisection-reconnection branch swapping algorithm in PAUP version 3.0q (Phylogenetic Analysis Using Parsimony, Swofford 1989) which is a heuristic method, and does not guarantee to find the most parsimonious cladogram. To increase the probability of finding all most parsimonious trees

the analysis was performed 100 times with the taxa entered in random order. Trees were rooted using the diatom Phaeodactylum, the ascomycete Saccharomyces (Rubstov et al. 1980), and the brown alga Costaria (Bhattacharya and Druehl 1988) as outgroups. Alternative phylogenetic hypotheses were tested by enforcing topological constraints and comparing the change in the number of evolutionary steps. Forty-four non-molecular characters were compiled from the primary literature (see Chapter One for characters and their descriptions) and included in a data set with the sequence data. The combined data were analyzed in the same way as the sequence data alone. All characters were considered to be multistate and unordered.

The Decay Index (DI; Mishler et al. 1991, Donoghue et al. 1992) measures the relative amount of character support for a node. The DI was determined by retaining all trees 5 steps longer than the most parsimonious tree using PAUP's heuristic search option with tree bisection-reconnection branch swapping. The Filter Trees option was used to identify trees one through five steps longer than the

most parsimonious tree. At each step a strict consensus of the filtered trees was constructed. As parsimony was relaxed, the number of steps it took to collapse a particular branch to a polychotomy in the consensus tree was the DI value assigned to that branch.

The degree of randomness in the molecular data was tested with the g_1 analysis (Hillis and Huelsenbeck 1992). A tree-length distribution, and the g_1 skewness statistic, were generated from 10,000 random trees with the random-search option in PAUP.

The ACCTRAN and DELTRAN optimizations of the characters for the most parsimonious tree were used in separate analyses to count the number of transitional and transversional changes.

Transversional data were analyzed using the equate command in PAUP to equate A=G and C=T. In other words, the recoding resulted in specifying purines and pyrimidines, so that any change from a purine to purine or a pyrimidine to pyrimidine was masked. The data set was then run with the same commands as for the unaltered data.

Three separate tests using Evolutionary Parsimony (Lake 1987) were employed to examine the positions of Pedinomonas minutissima, Micromonas and Mantoniella, and Pyramimonas and Mesostigma. Each test requires specifying four groups of taxa, and every possible topology of four taxon statements is examined. The test for P. minutissima specified 1) P. minutissima, 2) the Charophyceae and Land Plants, 3) Costaria and Phaeodactylum, and 4) Saccharomyces. The test for Micromonas and Mantoniella specified 1) Micromonas and Mantoniella, 2) P. minutissima, 3) the Charophyceae and Land Plants, and 4) the outgroup taxa. The test for Pyramimonas and Mesostigma specified 1) the two Pyramimonas species, 2) Mesostigma, 3) the Charophyceae and Land Plants, and 4) the outgroup taxa and P. minutissima.

A maximum likelihood analysis of the data was performed using the DNAML program in PHYLIP ver. 3.2 (Felsenstein 1989). The Frequencies (F) option was specified to calculate the empirical base frequencies in the data set. These frequencies are used to calculate the probabilities of each type of base substitution. The default setting of the Categories

(C) option was chosen, which assumes that all base positions have an equal relative rate of change. The transition/transversion ratio was set at 2.0.

RESULTS AND DISCUSSION

Phylogenetic analysis of the sequence data alone generated a single most parsimonious cladogram (Fig. 3.1, length = 1352 steps, consistency index = 0.516, retention index = 0.561). Pleurastrophyte taxa are on the same lineage as chlorophyte taxa and not that of the ulvophyte taxa. These results are more consistent with the classification proposed by Mattox and Stewart (1984), who considered the Pleurastrophyceae and Chlorophyceae to be sister classes, than with the classification of Sluiman (1989), who considered the pleurastrophytes to be more closely related to the Ulvophyceae. However, the sequence data do not support monophyly for either the Pleurastrophyceae or the Chlorophyceae, which is not consistent with the Mattox and Stewart (1984) classification.

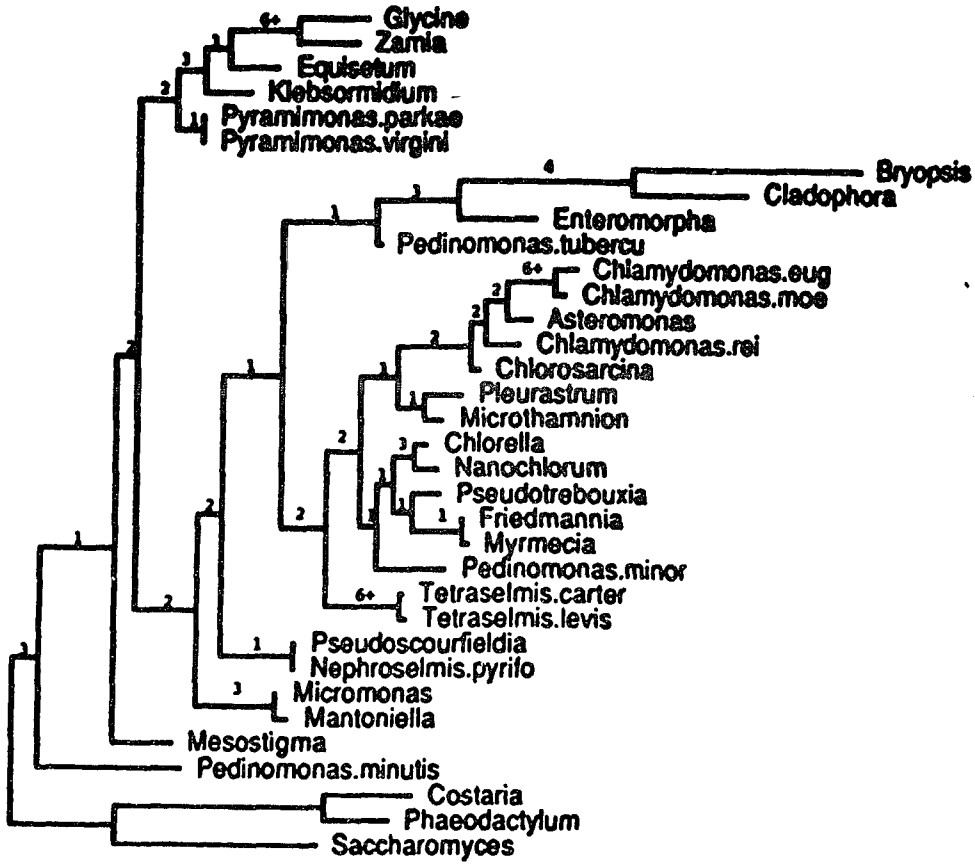

Figure 3.1. The most parsimonious cladogram generated from the sequence data alone. Length = 1352, Consistency Index = 0.516, Retention Index = 0.561. Numbers above the branches are Decay Index values for the nodes.

Within the Pleurastrophyceae + Chlorophyceae clade the pleurastrophycean taxa tend to form a grade below the chlorophycean taxa. The sarcinoid pleurastrophyte, Chlorosarcina, is the sister taxon to a clade of chlorophyte flagellates, which includes Chlamydomonas and Asteromonas. The filamentous pleurastrophytes, Pleurastrum and Microthamnion, form a clade that is sister to the clade of Chlorosarcina + Chlorophyte flagellates. The coccoid chlorophyte taxa Chlorella and Nanochlorum form a sister clade to the sarcinoid pleurastrophytes Myrmecia, Friedmannia, and Pseudotrebouxia. The Tetraselmis lineage is the basal divergence in the Pleurastrophyceae + Chlorophyceae clade.

The position of Tetraselmis on a clade of pleurastrophytes and chlorophytes supports the hypotheses of Mattox and Stewart (1984) and O'Kelly and Floyd (1984) that Tetraselmis is related to the Pleurastrum lineage because both groups share a metacentric mitotic spindle. It does not support the classifications of Moestrup and Throndsen (1988) and Melkonian (1990) who considered Tetraselmis to be closely related to the prasinophyte taxa Nephroselmis

and Pseudoscourfieldia because of their similar surface scale morphology.

The sister relationship between Friedmannia and Myrmecia supports the view of Deason (1989) that these two genera are closely related. Deason (1989) suggested that Friedmannia should be included in Myrmecia because of their similar plastid morphology and zoospore ultrastructure. Friedmannia is not on the Ulvophycean lineage, as suggested by Lokhorst (1984).

The cladistic analysis of the sequence data indicates that the Prasinophyceae is not monophyletic. Indeed, prasinophyte taxa appear on the Pleurastrophyceae + Chlorophyceae clade, the Ulvophyceae clade, and the Charophyceae + Land Plant clade. These results support the contention of Mattox and Stewart (1984) that the group is likely not monophyletic. Furthermore, the results are consistent with the cladistic analysis of Mishler and Churchill (1985), which found no uniquely derived characters to support monophyly for the Prasinophyceae. The evolutionary diversity of the prasinophyte taxa is not consistent with the views of

Moestrup and Throndsen (1988) and Melkonian (1990) that the Prasinophyceae is monophyletic.

Within the prasinophytes several relationships are consistent with prior taxonomic treatments. Both Pseudoscourfieldia and Nephroselmis have similar cell and flagellar scales (Moestrup and Throndsen 1988), and their sister relationship is consistent with the Chlorodendraceae of Moestrup and Throndsen (1988) and the Pseudoscourfieldiales of Melkonian (1990). The clade formed by Micromonas and Mantoniella is consistent with the Mamiellaceae sensu Moestrup and Throndsen (1988) and the Mamiellales sensu Melkonian (1990). It also corroborates studies of the photosynthetic pigments (Ricketts 1970, Foss et al. 1984, Wilhelm et al. 1986, Rowan 1989) and of the light harvesting pigment/protein complex (Fawley et al. 1986).

The morphology of the ancestral green flagellate has been a matter of much speculation. O'Kelly and Floyd (1984) suggest the ancestral green flagellate was heavily invested with surface scales and had a prominent flagellar pit, which they believe may have been a vestigial feeding apparatus. They propose

that the ancestral flagellate resembled a Pyramimonas-like or Halosphaera-like flagellate. Melkonian (1990) suggested that the ancestral flagellate was structurally simple with an asymmetric flagellar root; and he proposed that the ancestral flagellate resembled members of the Mamiellales (Mantoniella and Micromonas in this analysis), which have an asymmetric flagellar root with only two microtubular roots associated with only one flagellum. Moestrup (1991) proposed that the ancestral green flagellate resembled Pedinomonas, in which the nuclear membrane remains intact during mitosis, the telophase spindle is persistent, and the eyespot divides during cytokinesis.

The sequence data indicate great evolutionary diversity among Pedinomonas species. Pedinomonas minor is on a clade with pleurastrophyte and chlorophyte taxa; Pedinomonas tuberculata is on a clade with ulvophycean taxa; and Pedinomonas minutissima is the most basal green alga in the analysis. The evolutionary diversity of Pedinomonas does not support the concept of the Pedinomonadinae sensu Ettl (1966), the Pedinomonadales sensu

Melkonian (1990) or the Pedinophyceae sensu Moestrup (1991). The morphological and ultrastructural similarity among Pedinomonas species may be due to the retention of primitive features of the flagellar apparatus, or possibly due to the reduction of the flagellar apparatus to some sort of "functional unit," so that the different Pedinomonas species have converged on a similar morphology. The freshwater flagellates P. minor and P. tuberculata have been studied ultrastructurally (Moestrup 1991). Preliminary unpublished electron microscopic observations on the marine flagellate P. minutissima indicate that it does not resemble ultrastructurally the other Pedinomonas species; it is likely not a green alga, but further ultrastructural analysis is necessary before an unambiguous identification can be made (Moestrup, personal communication). Clearly, further ultrastructural study of Pedinomonas, particularly of the marine species, is necessary.

Pyramimonas and Mesostigma occupy basal positions among the green algae. Pyramimonas is on the Charophyceae + land plant lineage, and Mesostigma is basal to all the green algae in the study. These

Table 3.2. Increases in the length of user defined trees compared to the most parsimonious tree.

Saccharomyces sister to the chlamydomonads	+ 74
Monophyletic Chlorophyceae	+ 2
Monophyletic Pleurastrophyceae sister to the Chlorophyceae	+ 8
Monophyletic Pleruastrophyceae sister to the Ulvophyceae	+ 20
Chlorodendrales	+ 28
Pedinophyceae sister to the Chlorophyceae and Pleurastrophyceae clade	+ 8
Pedinophyceae sister to the Ulvophyceae	+ 20
Pedinophyceae basal in cladogram	+ 60
Pyramimonas basal in cladogram	+ 6
Micromonas and *Mantoniella* basal in cladogram	+ 2

results support the idea that a Pyramimonadales-like flagellate may be ancestral to the green algae (Norris 1980, O'Kelly and Floyd 1984, van den Hoek et al. 1989).

User Defined Trees. Alternative hypotheses of phylogeny were tested by comparing the lengths of user-defined trees with the length of the most parsimonious tree (Table 3.2). Longer trees have less support from the data than shorter trees. For comparisons, a member of the outgroup, Saccharomyces, was moved to the base of the chlamydomonad lineage. The resulting cladogram is 74 steps longer than the most parsimonious cladogram. Thus, hypotheses of ingroup relationships that approach lengths that are 74 steps longer than the most parsimonious tree are as poorly supported as the hypothesis that Saccharomyces is the sister taxon to Chlamydomonas.

The most parsimonious arrangement of the sequence data does not support the monophyly of the Chlorophyceae. Making the chlorophyceae monophyletic by moving the Chlorella + Nanochlorum clade to the

base of the chlamydomonad lineage increases the tree length by 2 steps.

In addition, the sequence data do not support monophyly of the Pleurastrophyceae. The classification of Sluiman (1989) places the pleurastrophyte taxa on the Ulvophyceae lineage because both groups share a counter-clockwise basal body orientation. Mattox and Stewart (1984) considered the Pleurastrophyceae and the Chlorophyceae sister classes because they both share a phycoplast microtubule arrangement during cytokinesis. Making the Pleurastrophyceae monophyletic and sister to the Chlorophyceae increases the tree length by eight steps. Making the Pleurastrophyceae monophyletic and sister to the Ulvophyceae increases the tree length by 20 steps. Thus, the sequence data show more support for the hypothesis of Mattox and Stewart (1984) than for the hypothesis of Sluiman (1989).

Mattox and Stewart (1984) considered Tetraselmis to be related to pleurastrophyte taxa, but Moestrup and Throndsen (1988) and Melkonian (1990) considered Tetraselmis to be related to the prasinophyte genera

Nephroselmis and Pseudoscourfieldia. In the most parsimonious cladogram Tetraselmis is in a clade with the Pleurastrophyceae and the Chlorophyceae. Moving Tetraselmis to the Nephroselmis + Pseudoscourfieldia lineage increases the tree length by 28 steps. Thus, the classifications of Moestrup and Throndsen (1988) and Melkonian (1990) are not supported.

The hypothesis that Pedinomonas minor and P. tuberculata are most closely related to the Ulvophyceae based on similarities in the flagellar ultrastructure (Melkonian 1984, Melkonian 1990) was tested. When P. minor was made the sister taxon to P. tuberculata on the ulvophycean line the cladogram increased in length ten steps. When P. tuberculata was made the sister taxon of P. minor on the Pleurastrophyceae + Chlorophyceae clade the tree length increased only six steps. Thus, there is less support for a Pedinomonas + Ulvophyceae clade than for a Pedinomonas + Chlorophyceae + Pleurastrophyceae clade. The ultrastructural similarities of Pedinomonas and the Ulvophyceae may be due to symplesiomorphic characters.

The ancestral flagellate has been hypothesized to resemble a Pyramimonas-like flagellate (O'Kelly and Floyd 1984, Floyd and O'Kelly 1990), a Mamiellales-like (Mantoniella or Micromonas) flagellate (Melkonian 1990), and a Pedinomonas-like ancestor (Moestrup 1991). As a test of these three hypotheses the taxa of interest were moved to the most basal position among the green algae (except for Pedinomonas minutissima). Moving the Pyramimonas clade from the Charophyceae lineage to the basal position among the green algae increases the tree length six steps. Moving the Micromonas + Mantoniella clade to the basal position increases the tree length by two steps. Moving a Pedinomonas minor + P. tuberculata clade to the base of the green algae increases the length of the tree by 60 steps. Thus, the sequence data offer greater support for the hypothesis of Melkonian (1990) that the ancestral flagellate was a Mamiellales-like flagellate. The hypothesis of a Pyramimonas-like ancestor (O'Kelly and Floyd 1984, Floyd and O'Kelly 1990) has slightly less support. Clearly, the hypothesis of an

ancestral Pedinomonas-like ancestor has little support from the sequence data.

Decay Index. The Decay Index (DI; Mishler et al. 1991, Donoghue et al. 1992) measures the amount of character support for a node by sequentially relaxing parsimony, constructing strict consensus trees of the cladograms one, two, three, etc. steps longer than the most parsimonious, and observing the number of steps required to collapse a particular node (Fig. 3.1). There were 12,563 trees five steps longer than the most parsimonious. Of these trees, sixteen were one step longer, 109 were two steps longer, 536 were three steps longer, 2562 were three steps longer, and 9439 were four steps longer than the most parsimonious. Relationships among the pleurastrophycean taxa are only weakly supported, with DI values of one or two, which is a result corroborated by user-defined tree experiments. Likewise, relationships among prasinophycean taxa have relatively low DI values. The sister taxa relationship of Pseudoscourfieldia and Nephroselmis collapses in only one step. The position of the

Pyramimonas species as sister to the Charophyceae and land plants collapses in two steps. The position of Pedinomonas minutissima is stable in trees up to three steps longer than the most parsimonious. The relationships with the greatest support are the clade of the two Tetraselmis species, the clade of the two Chlamydomonas species, and the clade of Glycine and Zamia, which were present in trees that were 5 steps longer than the most parsimonious tree.

The assumptions of the Decay Index have not been completely explored. One advantage the Decay Index has over bootstrapping methods is that it uses all the available data, rather than a randomly selected subset of the data. However, employment of strict consensus trees to calculate the DI values may lead to misleading conclusions. For example, if the position of a single taxon is poorly supported, that is, if moving the taxon around in the cladogram requires an increase in length of only a few steps, then a strict consensus tree may show little resolution, and the DI values may be low, even though relationships among the other taxa may be well supported. For this reason, the Decay Index should

be considered a conservative estimate of the amount of character data supporting a node.

Skewness test. The skewness test (Hillis and Huelsenbeck 1992) was used to examine the degree of randomness in the molecular data. The method is based on the observation that random data sets produce tree-length distributions that are symmetrical, whereas data sets with phylogenetic signal produce tree-length distributions skewed to the left. For the molecular data, the tree-length distribution of 10,000 random trees showed a statistically significant skew to the left. The skewness statistic, g_1, was calculated to be -0.59, which is less (i.e., more negative) than the critical value (P=0.01) of g_1 = -0.09 provided in Hillis and Huelsenbeck (1992) for data sets with four-state character data, ≥ twenty-five taxa, and 250-500 informative characters. Thus, the molecular data set shows a high level of signal, presumably phylogenetic.

Transition:Transversion ratio. The number of transitional and transversional changes was calculated using the ACCTRAN and DELTRAN optimization of the characters on the most parsimonious cladogram. Transitional changes were approximately twice as numerous as transversional changes (Table 3.3). The ratio is slightly higher than for Ulvophycean taxa (1.43:1, Zechman 1992) and for seed plants (1.67:1, Hamby 1990). However, Hamby (1990) and Zechman (1992) used a different method to calculate the transition:transversion ratio. In their alignments, they compared the variable base positions that had only purines with those that had only pyrimidines. Because their analyses were not based on a specific hypothesis of evolution but instead on the alignment itself, they are not able to take into account the possibility of convergence or reversals. Therefore, the ratios they calculated may be an underestimate of the actual value.

Transversion parsimony. Because transitions are more frequent than transversions it is possible that transitional data may be noisier than transversional

Table 3.3. The number of transitional and transversional changes provided by the ACCTRAN and DELTRAN optimizations for the most parsimonious tree of the sequence data. DELTRAN values are in parentheses. Transitional changes are A <-> C and G <-> T. Transversional changes are A or C <-> G or T.

CHANGES FROM \ CHANGES TO	G	A	T	C
G	–	216 (202)	129 (126)	131 (136)
A	208 (217)	–	93 (89)	98 (96)
T	101 (101)	114 (119)	–	282 (296)
C	102 (96)	75 (67)	204 (207)	–

data. In addition, the signal from the more conservative transversional data may be masked by the noisier transitional data. Indeed, it is possible that the noisy data may result in the spurious or artificial resolution of certain relationships in the most parsimonious cladogram. Furthermore, the more conservative transversional data may be more useful in resolving relationships at the basal nodes and less useful at resolving relationships at the terminal nodes.

On the other hand, it is possible that transitional changes, though more frequent, are not so frequent as to contribute excessive noise to the data set. And the exclusion of the transitional data may result in the loss of resolution at both basal and terminal nodes.

Cladistic analysis of the transversion data yielded 918 most parsimonious cladograms of length 779 steps and C.I. of 0.616. The strict consensus tree of these cladograms is shown in Figure 3.2. Relationships among the pleurastrophyte and chlorophyte taxa are ambiguous; and in the most parsimonious cladogram of the unaltered data set,

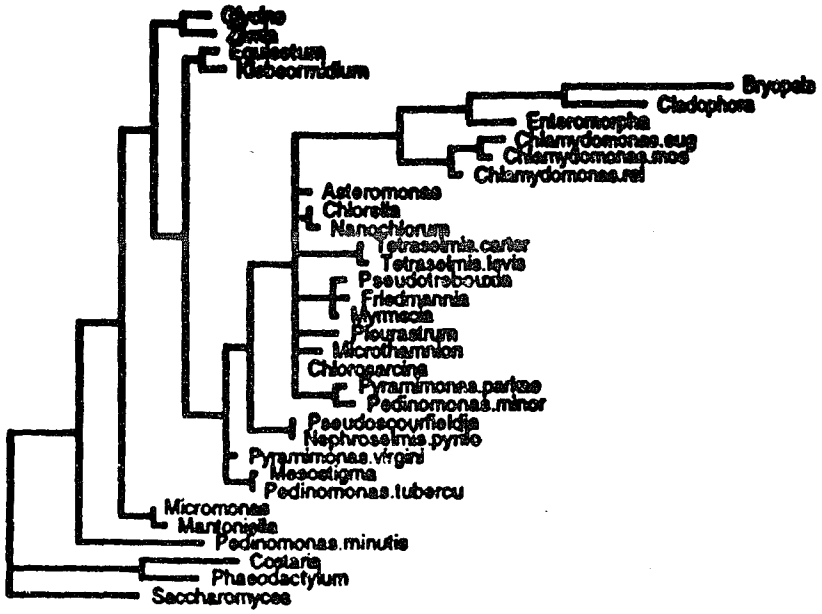

Figure 3.2. Strict consensus tree of 918 most parsimonious cladograms from the transversion data. Length = 779, Consistency Index = 0.616.

these relationships were shown by the user tree topologies and the Decay Index test to be weakly supported. However, several of the basal relationships among the major groups are not consistent with the most parsimonious tree of the unaltered data. The Ulvophyceae is the sister group to the genus Chlamydomonas, a relationship that is not supported when transitional data are considered. Nor is it supported in other molecular (Zechman 1992) or morphological studies (Mattox and Stewart 1984, Mishler and Churchill 1985). Another problematic relationship seen in the transversion data cladogram is the non-monophyly of the vascular plants. The Charophyceae and Land Plants form two clades, with the vascular plant Equisetum in a clade with the charophyte alga Klebsormidium separated from the other vascular plants Glycine and Zamia. This result is inconsistent with other molecular (Hamby 1990) and morphological studies (Mishler and Churchill 1985) which support monophyly for the vascular plants.

The results of the transversion data analysis indicate that transitional data are useful even at the more basal divergences. Apparently, the rate of

transitional change is not so fast that it obscures basal relationships. Indeed, the use of transversional data alone did not produce results consistent with commonly accepted hypotheses of green plant evolution.

Evolutionary parsimony. In the case where different lineages have greatly unequal rates of evolution, such as the model proposed by Felsenstein (1978), parsimony analysis may fail to find the correct phylogeny (Felsenstein 1978, Li et al. 1987). Evolutionary Parsimony is a method of analysis designed to find the correct phylogeny under the model proposed by Felsenstein (Lake 1987). The method compares the three possible unrooted relationships of four taxa; and it assumes that the rate of transitional changes is high and obscures the phylogenetic signal of the transversional changes. Nucleotide sites are scored as supporting a particular relationship if they include two identical purines and two identical pyrimidines (e.g. GGTT or ACAC) or two non-identical purines and two non-identical pyrimidines (e.g. ACGT). Sites scored as

counter-supporting a topology include two identical purines and two non-identical pyrimidines (e.g. GGCT) or two non-identical purines and two identical pyrimidines (e.g. GATT). By summing the number of supporting nucleotide sites and subtracting the number of countersupporting sites a score for each topology is generated. A topology with a score significantly higher than zero based on a X^2 test is supported.

To test whether unequal rates of evolution have perhaps obscured relationships among the basal lineages of the most parsimonious cladogram, three analyses using evolutionary parsimony were conducted to test the positions of Pedinomonas minutissima, Micromonas and Mantoniella, and Pyramimonas and Mesostigma. In none of the tree tests was a topology found that had a score significantly above zero at the α=0.05 level; however, in all cases the relationship with the highest absolute score was also the relationship found in the most parsimonious cladogram. Evidently, there are not enough sequence data to recover a significant score for any topology. But the trend in the data supports the relationships

found in the most parsimonious cladogram, even under a model which assumes greatly unequal rates in different lineages.

Maximum likelihood. The maximum likelihood approach to phylogeny reconstruction assumes a specific model of evolution, such as specific probabilities of nucleotide changes for specific base positions, and then calculates the probability of occurrence of the observed data given a phylogenetic tree. Multiple trees are examined and compared, and the tree giving the greatest likelihood of the data is considered the best supported hypothesis of phylogeny under the specified model (Felsenstein 1984).

The model specified in the DNAML program of PHYLIP is an equilibrium model which assumes an infinite pool of nucleotides (Felsenstein 1989). The ratio of nucleotides in the pool was calculated with the F option, which uses the empirical base frequencies in the data set. The empirical frequencies are used to calculate the prior probabilities of each type of base substitution. All

base positions were assumed to have an equal relative rate of change (the default setting of the C option). The transition/transversion ratio was set at 2.0.

In the maximum likelihood tree (Fig. 3.3), as in the parsimony tree, the Chlorophyceae and the Pleurastrophyceae form a clade, however neither the Chlorophyceae nor the Pleurastrophyceae are monophyletic. The uninucleate ulvophyte Enteromorpha is more closely related to the chlorophycean and Pleurastrophycean taxa than to the siphonous ulvophytes. This result supports the hypothesis of Zechman, et al. (1990) that the Ulvophyceae are not monophyletic. The Charophyceae and land plants form a monophyletic group that emerges from a relatively basal node. The prasinophyte taxa are not monophyletic. Several prasinophyte taxa represent basal divergences. Pedinomonas minutissima is the basal green alga, Micromonas and Mantoniella form the next diverging clade, and basal to the rest of the green algae. Mesostigma is the next diverging alga.

Problematic positions of some prasinophytes are also seen. Pyramimonas virginica is basal to the

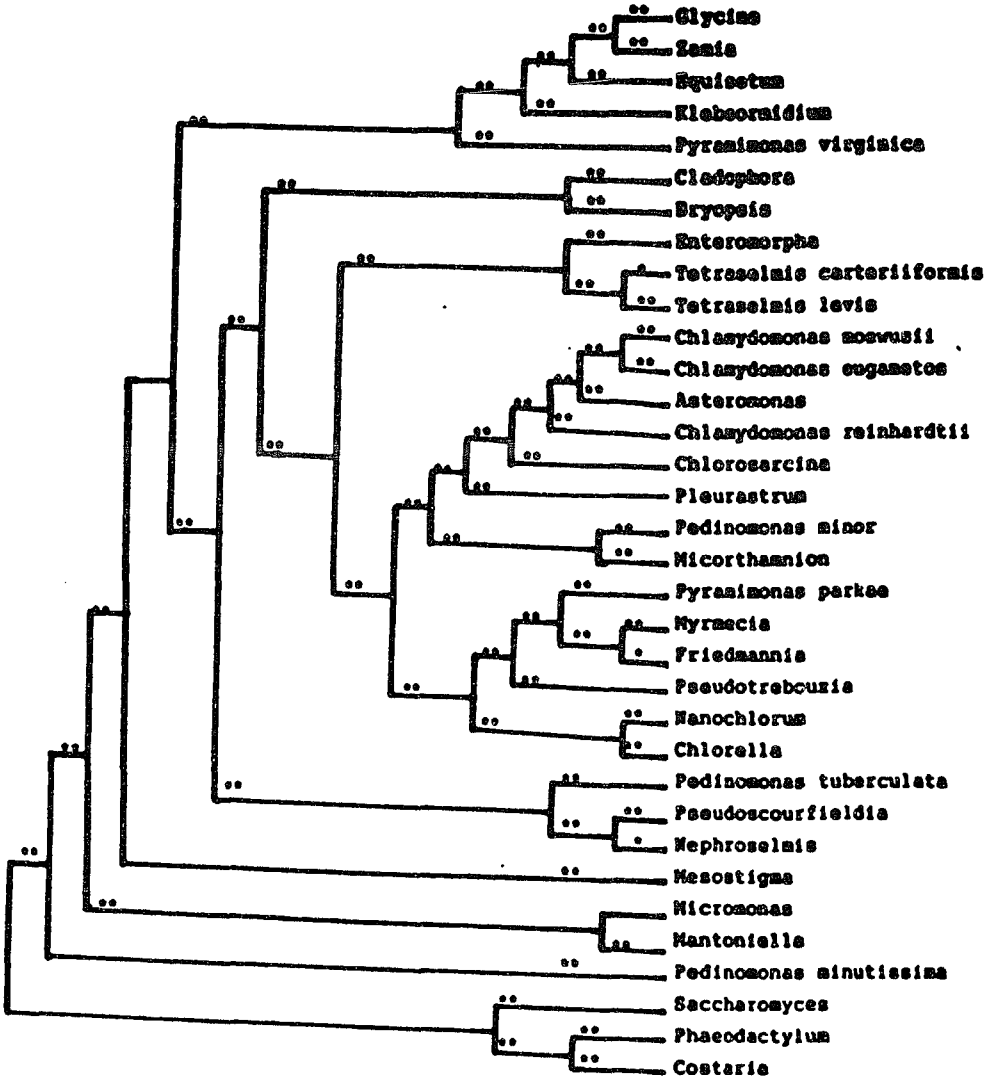

Figure 3.3. The maximum likelihood tree of the sequence data alone. Branch lengths significantly above zero are denoted by astersiks (** = $P < 0.01$, * = $P < 0.05$).

Chlorophyceae/land plant clade while Pyramimonas parkae is sister to the sarcinoid plerastrophyte taxa Myrmecia and Friedmannia. Pedinomonas minor is in the Chlorophyceae/Pleurastrophyceae clade, and Pedinomonas tuberculata is sister to the Nephroselmis and Pseudoscourfieldia clade.

Because maximum likelihood methods require such a specific model of evolution, their use for phylogenetic analysis is probably limited. If the evolutionary model is appropriate, then the method is very powerful; however, rarely is one certain that the model is correct. Therefore, the results of the maximum likelihood analysis should be interpreted with caution.

Combined data set. Cladistic analysis of the sequence data and the 44 ultrastructural and biochemical characters generated thirteen most parsimonious cladograms (length = 1446, CI = 0.508, RI = 0.552). The majority rule consensus tree of the thirteen cladograms is presented in Figure 3.4. Much of the disagreement among the cladograms is seen in the relationships the chlorophycean and

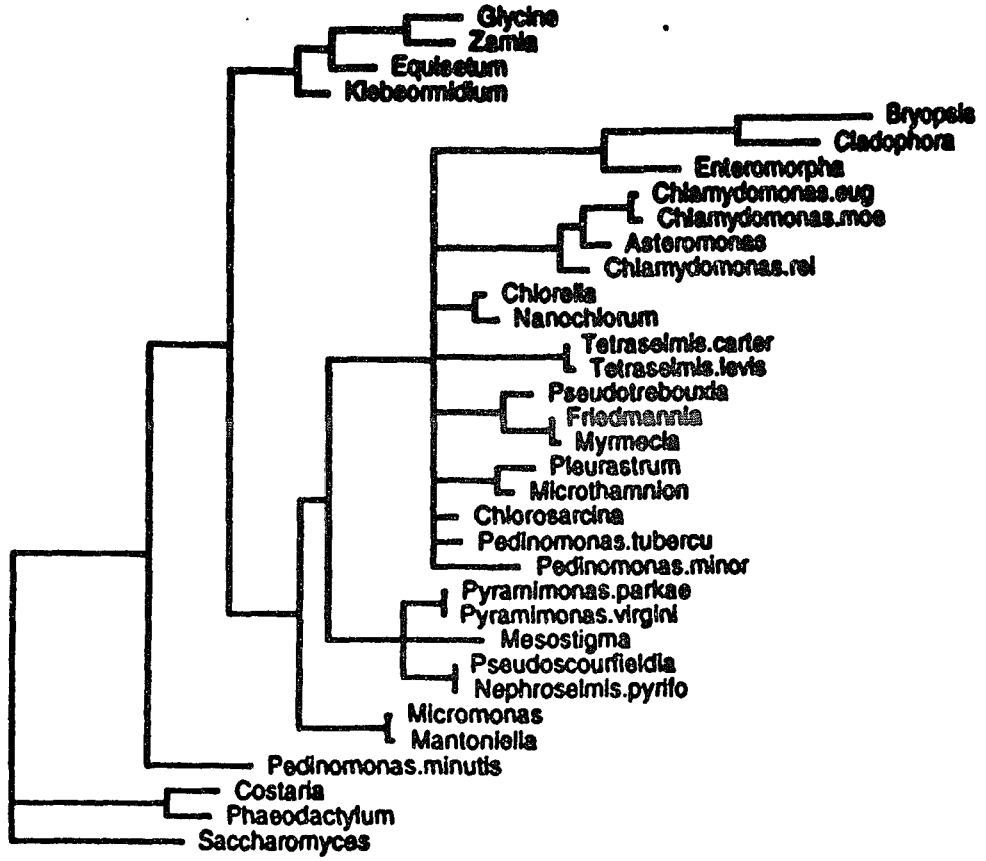

Figure 3.4. Strict consensus tree of the 13 most parsimonious cladograms generated from the combined molecular and non-molecular data. Length = 1446, Consistency Index = 0.508.

pleurastrophycean taxa. In a majority of the cladograms the Pleurastrophyceae and Chlorophyceae form a clade, although monophyly for either class is ambiguous. Certain clades are supported in both the combined data and in the sequence data only; for example, the clades of the filamentous pleurastrophytes (Pleurastrum and Microthamnion), the sarcinoid pleurastrophytes (Friedmannia, Myrmecia, and Pseudotrebouxia), the Tetraselmis species, the Chlamydomonad taxa, and the coccoid chlorophytes (Chlorella and Nanochlorum) are found also in the cladogram from the sequence data alone. However, much of the disagreement among the cladograms is found in the relationships among these clades. These relationships were shown to be poorly supported in the Decay Index test and the user-defined trees from the sequence only.

One important difference between the cladogram from the sequence data alone and the cladograms from the combined data is the positions of the hypothesized ancestral flagellates: Pedinomonas minor and P. tuberculata, Mesostigma and the Pyramimonas species, and Mantoniella and Micromonas.

The cladogram generated from the sequence data alone placed Pedinomonas minor with pleurastrophycean taxa and P. tuberculata with ulvophycean taxa. The cladogram generated with the combined data places P. minor and P. tuberculata in the clade with the Ulvophyceae, the Pleurastrophyceae, and the Chlorophyceae. These results do not support the hypothesis that Pedinomonas diverged early from the green algae (Moestrup 1991). In the cladogram of sequence data alone Pyramimonas and Mesostigma were relatively basal among the green algae. When the molecular and non-molecular data are combined Pyramimonas and Mesostigma move from a basal position to a more derived position on the Nephroselmis and Pseudoscourfieldia clade, a relationship consistent with the Chlorodendrales sensu Moestrup and Throndsen (1988). These results do not support the hypothesis that the ancestral flagellate was Pyramimonas-like (O'Kelly and Floyd 1984). In the cladogram from the combined data, the Micromonas and Mantoniella clade move to the most basal position among the green algae (except for Pedinomonas minutissima), which supports

the hypothesis of Melkonian (1990) that the ancestral flagellate was likely a Mamiellales-like flagellate.

CONCLUSION

The fossil record for the green algae extends back 900-1000 million years (Tappan 1980). Despite the great antiquity of the green algae, ribosomal RNA sequence data are useful for resolving relationships among the major lineages. The greater rate of transitional changes compared to transversional changes (approximately a two to one ratio) does not render the rRNA data inappropriate for this phylogenetic level. Indeed, the g_1 analysis indicates a strong phylogenetic signal in the data. Furthermore, the data are robust even under different models of evolution, such as Evolutionary Parsimony and maximum likelihood.

The sequence data support many aspects of the Mattox and Stewart (1984) classification based on morphological and ultrastructural information. Pleurastrophycean taxa are more closely related to

chlorophycean taxa than they are to ulvophycean taxa. In addition, Tetraselmis is more closely related to pleurastrophycean taxa, as suggested by Mattox and Stewart (1984) and O'Kelly and Floyd (1984), than to Nephroselmis and Pseudoscourfieldia, as suggested by Moestrup and Throndsen (1988) and Melkonian (1990). Neither the Pleurastrophyceae nor the Chlorophyceae were monophyletic in the cladistic analysis of the sequence data; however, the tests with the user tree topologies showed that the monophyly for both classes required only a few steps longer than the most parsimonious tree. Additional data from future studies of may resolve the issue of monophyly of these classes.

The sequence data indicate that the Prasinophyceae is not monophyletic. These results are consistent with the views of Mattox and Stewart (1984) and the cladistic analysis of green algal classes by Mishler and Churchill (1985). However, the results are not consistent with the classifications of Moestrup and Throndsen (1988) and Melkonian (1990). These two classifications greatly emphasized the taxonomic importance of cell and

flagellar surface scales. The great evolutionary diversity among prasinophyte taxa indicates that surface covering may be overemphasized in constructing taxonomic schemes for these taxa.

The cladistic analysis of the morphological data alone (see Chapter One) supported the hypotheses that the ancestral green flagellate was Mamiellales-like (Mantoniella and Micromonas) or Pedinomonas-like (Melkonian 1990, Moestrup 1991), but provided less support for a Pyramimonadales-like ancestor (O'Kelly and Floyd 1984). The sequence data alone support a Mamiellales-like ancestor or a Pyramimonadales-like ancestor, but do not support a Pedinomonas-like ancestor. The analysis of the combined data set resolved the ambiguity present in each data set alone, and provides support for the hypothesis of a Mamiellales-like ancestor. Further ultrastructural and molecular examination of Pedinomonas minutissima will likely prove useful in studies of the origin of the green algae.

Some relationships, particularly those in the Pleurastrophyceae + Chlorophyceae clade, are not well supported in the sequence data. Clearly more

systematic studies, both morphological and molecular, are needed to resolve these uncertainties. Ultrastructural studies of mitosis and cytokinesis within the prasinophytes may provide much phylogenetic information. Molecular data sets of conserved nuclear genes of the flagellar and cytoskeletal components may resolve relationships among the major lineages. In addition, independent molecular data sets of chloroplast and mitochondrial genes should contribute significantly to our understanding of molecular evolution in the green algae.

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CHAPTER FOUR

INTRAGENERIC RELATIONSHIPS OF TETRASELMIS INFERRED FROM ITS1, ITS2, AND 5.8S rDNA SEQUENCE DATA

INTRODUCTION

Molecular studies of the green algal flagellate Chlamydomonas have revealed considerable variation among species (Jupe et al. 1988, Buchheim et al. 1990). Indeed, cladistic analysis of the molecular data show that there are many lineages within Chlamydomonas and that the genus is not monophyletic (Buchheim et al. 1990). In addition, molecular studies of species of the colonial green flagellate Pandorina indicate extensive molecular variation among lineages and mating types. Unfortunately, additional intrageneric studies of green algal flagellates are few, so that it is not known how general the phenomenon of high intrageneric molecular variation among green flagellate species is. A preliminary molecular study of the morphologically diverse flagellate genus Tetraselmis was conducted to assess the level of sequence divergence among lineages within the genus.

Tetraselmis Stein is a genus of green flagellates that includes many marine, and a few

freshwater, species. Species of Tetraselmis have been distinguished on the basis of cell symmetry, theca morphology, position of the eye spot, and characteristics of the chloroplast and pyrenoid (Butcher 1959). Melkonian (1979) studied the ultrastructural features of the flagellar apparatus of Tetraselmis cordiformis and found many similarities between it and the genus Platymonas G.S. West. Norris et al. (1980) reviewed both published information and their own ultrastructural observations of Tetraselmis and the morphologically similar genera Platymonas, Prasinocladus Kuckuck, and Aulacochlamys Margalef, all of which they considered synonymous with Tetraselmis in their revision of the genus.

Hori et al. (1982) described four subgenera of Tetraselmis distinguished by ultrastructural features of the chloroplast and pyrenoid, although possible evolutionary relationships among the subgenera were not discussed. The subgenus Tetraselmis is distinguished by a pyrenoid which is traversed by several branching cytoplasmic channels entering the matrix from all directions (Hori et al. 1982). The

cytoplasmic channels contain an electron dense material that is separated from the rest of the cytoplasm by a single membrane. The subgenus Prasinocladia is distinguished by a pyrenoid with a single large cavity which is filled by a lobe of the nucleus (Hori et al. 1983). The subgenus Tetrathele contains a pyrenoid that has a large cavity containing a lobe of cytoplasm, and having several small channels that traverse the pyrenoid matrix (Hori et al. 1982). The subgenus Parviselmis contains a pyrenoid similar to that in the subgenus Tetrathele, but the pyrenoid is smaller, and the small channels end blindly in the matrix (Hori et al. 1986).

In a class level study of the green algae, Kantz et al. (1990) compared partial sequences of the large and small subunits of the ribosomal RNA. The two Tetraselmis species included in the analysis had nearly identical sequences, even in the highly variable regions. Therefore, to resolve relationships within the genus Tetraselmis, an even more variable region was selected for study. This was the ribosomal internal transcribed spacer (ITS) region,

which includes the sequences 3' to the 18S rRNA coding region, or ITS1, the 5.8S rRNA coding region, and the region 3' to the 5.8S region, or ITS2 (White et al. 1990). The only other study of algal evolution using these variable regions is that described in the published abstract of Bakkar et al. (1990), who used the 5.8S rDNA and ITS sequences in a study of disjunct populations of the green macroalga Cladophora albida.

We used the polymerase chain reaction (PCR) amplification method (Innis et al. 1990) to obtain microgram quantities of DNA for sequencing. Because only small amounts of starting material were needed, large scale culturing of algae was unnecessary.

MATERIALS AND METHODS

Cultures Used

The Tetraselmis strains used in the current study (Table 4.1) were strains listed by Norris et al. (1980), Hori et al. (1982), Hori et al. (1983), and Hori et al. (1986) in the original descriptions

Table 4.1. List of *Tetraselmis* strains used in the study. Sources, subgeneric designations, starting wet weight, and total DNA yields are also provided.

Strain	Source	Subgenus	Wet Wt./Total DNA
Tetraselmis chuii Butcher	UTEX 232	Parviselmis	0.02 g / 40.4 µg
Tetraselmis levis Butcher	CCMP Platyl	Parviselmis	0.03 g / 53.4 µg
Tetraselmis striata Butcher	CCMP UW490	Parviselmis	0.04 g / 43.0 µg
Tetraselmis suecica Butcher	UTEX 2286	Parviselmis	0.01 g / 51.8 µg
Tetraselmis tetrahele Butcher	CCMP UW421	Tetrathele	0.03 g / 40.4 µg
Tetraselmis tetrahele Butcher	CCMP UW494	Tetrathele	0.03 g / 23.1 µg
Tetraselmis verrucosa Butcher	CCMP UW480	Prasinocladia	0.04 g / 47.1 µg
Tetraselmis astigmatica Norris et Hori	CCMP UW436	Tetraselmis	0.01 g / 36.7 µg

of the subgenera. Cultures were maintained in 5 ml aliquots of K or f/2 medium (Keller et al. 1987). Cultures were grown at 20° C under a 16:8 h LD cycle. Cells were harvested after 7-10 days.

Nucleic Acid Extraction

A procedure for the rapid extraction of DNA from microalgae was developed during this study, and is a refinement of the extraction protocol of Coleman and Grossman (1984). In addition to the Tetraselmis species used in the study, this method has proven successful for other green unicellular algae in the Pleurastrophyceae and Prasinophyceae. The DNA from Chlamydomonas reinhardtii (CC1952, Duke University Chlamydomonas collection) was provided by Dr. Mark Buchheim, Louisiana State University.

Solutions. The solutions required for the extraction include:

Stock solutions: 4.0 M NaCl
0.5 M Na-EDTA (pH 5.0-8.0)
1.0 M Tris HCl (pH 9.0)
20% (by volume) sodium dodecylsulfate (SDS)

50:50:1 phenol:chloroform:
isoamyl alcohol
Isopropanol or ethanol
at -20° C
1.0 M Tris-EDTA

Extraction Buffer: 12.5 ml of the 4.0 M NaCl stock
7.5 ml of the 0.5 M Na-EDTA
12.5 ml of the 1.0 M Tris-HCl
205.0 ml of sterile, distilled H_2O

Cell Concentration. The cells from one 5 ml aliquot of a 7-10 day culture were concentrated by centrifugation, and the culture medium decanted. The cells were resuspended in 0.5-1.0 ml extraction buffer to wash the cells of any remaining culture media, and the sample was transferred to a 1.5 ml microcentrifuge tube. The cells were pelleted by microcentrifugation and the wash removed. The cells were resuspended in 250 µl of the extraction buffer.

Cell Breakage. Several methods of cell breakage were tried. Adding enough SDS to form a 1-2% solution (2.5-5.0 µl of a 20% SDS solution to 250 µl of the extraction buffer), followed by sonication with short pulses (5 secs.) for up to one minute, was an efficient method of cell breakage for Tetraselmis. Care was taken to avoid prolonged sonication so that the DNA was not sheared excessively. To prevent overheating of the samples during sonication, samples were sonicated on ice.

The amount of cell breakage was determined microscopically by checking a drop of the homogenate for cellular debris. Once adequate cell breakage (>70%) was obtained, the cell debris was pelleted with microcentrifugation for about two minutes.

Nucleic acid isolation. An equal volume of a 50:50:1 phenol:chloroform:isoamyl mixture was added to the 250 µl of cell homogenate. Extraction then was done according to standard methods for nucleic acid isolation. The mixture was agitated by hand or by gentle vortexing for 2-4 minutes. It was then centrifuged for two minutes or until the aqueous and organic phases separated. The aqueous phase (top

layer) was pipetted off and placed in a clean microcentrifuge tube. The organic phase was discarded.

To the aqueous phase 250 µl of chloroform was added and agitated 2-4 minutes. It was centrifuged for two minutes until the phases separated. The aqueous phase was pipetted to another clean microcentrifuge tube and the organic layer discarded. To the aqueous phase 150 µl of chilled isopropanol or 500 µl of chilled ethanol was added, mixed gently, and placed at -20° C for about one hour to precipitate the DNA.

The sample was then centrifuged for ca. 2 minutes and the supernatant removed. The precipitated DNA was dried either by air or by vacuum. The nucleic acid pellet was resuspended in ca. 40 µl sterile H_2O or sterile 1 M Tris-EDTA buffer. The concentration of the DNA was determined by UV spectrophotometry (Table 4.1) and the integrity of the DNA by agarose gel electrophoresis.

PCR Protocol

Primers used in amplifying the Internal Transcribed Spacer region were TB5 and TB6 of White et al. (1990). Production of PCR-amplified linear DNA templates for direct sequencing followed the method of Kaltenboek et al. (1992).

Symmetric amplification. The symmetric amplification reaction mixture included 10-200 ng complex DNA, 20-50 pmoles of both primers, 50-100 µmoles of dNTPs, and 2.0 units Taq polymerase in a final volume of 100 µl.

The optimum temperature cycle for the symmetric amplification step was found to be 93° C for 3 minutes, 50° C for 1 minute, 72° C for 1.5 minutes (for 1 cycle); 93° C for 1 minute, 50° C for 1 minute, 72° C for 1.5 minutes (for 29 cycles); 72° C for 5 minutes; 4° C soak.

After the symmetric PCR amplification was finished the quality of the reaction was checked by running 10 µl of the symmetric PCR product and 2 µl of dye on a 1% agarose minigel at 50 mA and approx. 100-125 mV. The agarose gels were stained with a solution of 100 ml dH_2O and 15 µl ethidium bromide

for 15 minutes. Destaining was carried out in dH_2O for 15 minutes. The gel was then examined on a UV light box. The length of the symmetric product was ca. 650 bp.

The above method worked well for Chlamydomonas and all strains of Tetraselmis except Tetraselmis astigmatica of the subgenus Tetraselmis. Even less stringent conditions (lower annealing temperatures and temperature ramps at the annealing step) did not yield amplified product from T. astigmatica.

Asymmetric amplification. The asymmetric PCR amplification reaction mixture included 10 µl symmetric PCR product, 20-50 pmoles of one primer, 50-100 µmoles of dNTPs, and 2.0 units Taq polymerase in a final volume of 100 µl.

The optimum temperature cycle was 93° C for 3 minutes, 50° C for 1 minute, 72° C for 2 minutes (for 1 cycle); 93° C for 1 minute, 50° C for 1 minute, 72° C for 2 minutes (for 19-24 cycles); 72° C for 7 minutes; 4° C soak.

The quality of the single stranded PCR reaction was checked on 1% agarose gels using the same protocol as the symmetric reaction.

The above method worked well for all strains of Tetraselmis and the Chlamydomonas using the TB5 primer, but only worked for Chlamydomonas and Tetraselmis verrucosa using the TB6 primer.

Sequencing Protocol

For sequencing of the asymmetric PCR product, the single-stranded product was separated from unincorporated primers, dNTPs, and PCR reaction buffer salts using Millipore UFC3 TTK 00 (30,000 NMWL) filter cartridges. The filter cartridge chambers were first brought to full volume with autoclaved dH_2O (ca. 0.4 ml) and spun in an Eppendorf 5415C variable speed microcentrifuge at 4500 rpm (approximately 1800g) for about 1 minute. The asymmetric PCR product was added, the filter chamber was brought to full volume with autoclaved dH_2O, and was centrifuged for about 2 minutes until the volume was reduced to about 100 µl. The filtrate was removed, the filter chamber was brought to full volume with dH_2O, and spun until the volume was reduced to 100 µl. The washing procedure was repeated 6 times. On the last wash the volume was reduced to

50 µl and the sample was removed to a sterile microcentrifuge tube.

Sequencing of the single stranded, asymmetric PCR product followed the protocols provided with the Sequenase sequencing kit. Best results were obtained for Chlamydomonas, Tetraselmis verrucosa, T. striata. and T. levis using 5-10 pmol of sequencing primer per reaction, and dNTP labeling solutions were diluted 15-20 fold. ^{35}S-dATP-labeled reaction products were separated by 6% polyacrylamide-urea gel electrophoresis and visualized by autoradiography.

Tetraselmis chuii, T. suecica, T. tetrahele (UW421),and T. tetrahele (UW494) did not yield readable sequences with the above sequencing method. The autoradiographs showed many double bands and stops, indicating possible non-specific binding of the sequencing primer or polymorphism of the ITS types (Suh et al., in preparation). The double-stranded, symmetric PCR product was placed in a boiling water bath for 2 minutes, then immediately placed in an ice bath for 1 minute. The sequencing reactions were then carried out as above. This

method also worked with those taxa that did not produce asymmetric product using the TB6 primer.

Sequence alignment

Published sequences of the 5.8S rRNA from Saccharomyces cerevisiae (Rubin 1973), Triticum vulgare (MacKay et al. 1980, Wildeman and Nazar 1982), and Vicia faba (Tanaka et al. 1980, Nazar and Wildeman 1981) were obtained from GenBank to aid in alignment of the algal sequences, though they were not used in the subsequent phylogenetic analyses. The ITS1 and ITS2 sequences from Chlamydomonas reinhardtii were too different from the Tetraselmis sequences to align them with confidence, so they were not included in the subsequent analyses. However, the 5.8S sequence from Chlamydomonas reinhardtii aligned well with the Tetraselmis 5.8S sequences, so it was used to root the cladograms. The sequences were aligned using the GAP, LINEUP, and PRETTY programs in the University of Wisconsin Genetics Users Group software package (UWGCG version 6.1, Devereux et al. 1984) now known as the GCG package. Final alignments

were performed by hand. The final alignment was 482 bases in length (Appendix B).

Phylogenetic analysis

Aligned sequence data were analyzed using the Branch and Bound search option, which guarantees to find the most parsimonious cladogram, in PAUP version 3.0q (Phylogenetic Analysis Using Parsimony, Swofford 1989). All characters were considered to be multistate and unordered. Trees were rooted using the Chlamydomonas 5.8S sequence.

RESULTS AND DISCUSSION

One major drawback to molecular studies of microalgae is the need for growing algae in batch culture. Many molecular techniques require relatively large quantities of nucleic acid, necessitating the cultivation of algae in several

liters of media or more (Buchheim et al. 1990, Kantz et al. 1990). However, many microalgae do not grow well in batch culture, possibly due to the production of growth inhibitors (Ricketts 1974), or the activity of algal viruses (Melkonian 1982). Furthermore, batch culturing requires large amounts of space and time, and often the algae are difficult to maintain axenically.

An advantage of the nucleic acid extraction protocol used is that it requires small quantities (0.01 gm) of fresh tissue. Often the amount that a culture collection provides in a culture tube is sufficient for successful DNA isolation. In addition, the method requires only a few hours for processing, and it is carried out in microcentrifuge tubes, thus using a minimum of glassware. Routinely, 20-40 µg of total DNA is isolated from 0.01-0.04 gm (wet weight) of unicellular Chlorophyta with this method (Table 4.1). The PCR amplification protocols require nanogram quantities of DNA, thus 20-40 µg is sufficient for PCR use.

The symmetric amplification step for Tetraselmis astigmatica was not successful, even with less

stringent conditions. The reason for this is not known, but it is possible that one of the oligonucleotide primers was not annealing to the DNA template. Because sequence data were not obtained for T. astigmatica, a member of the subgenus Tetraselmis, it was not included in the phylogenetic analysis.

The sequences from the other seven Tetraselmis strains and from the Chlamydomonas reinhardtii 5.8S sequence yielded a final alignment of 482 base positions in length. To see if the 5.8S rDNA region (base positions 164-334) was variable enough to resolve relationships among the subgenera, a phylogenetic analysis of the 5.8S data only was conducted. A single most parsimonious cladogram resulted (length = 80 steps, CI = 0.862, and RI = 0.919) (Fig. 4.1) with resolution of one of the subgenera from the other two. Tetraselmis verrucosa of the subgenus Prasinocladia was basal to the Tetrathele/Parviselmis clade. However, there was no resolution among species of the Tetrathele and the Parviselmis subgenera. Hori et al. (1982) noted the structural similarity of the pyrenoids of these two

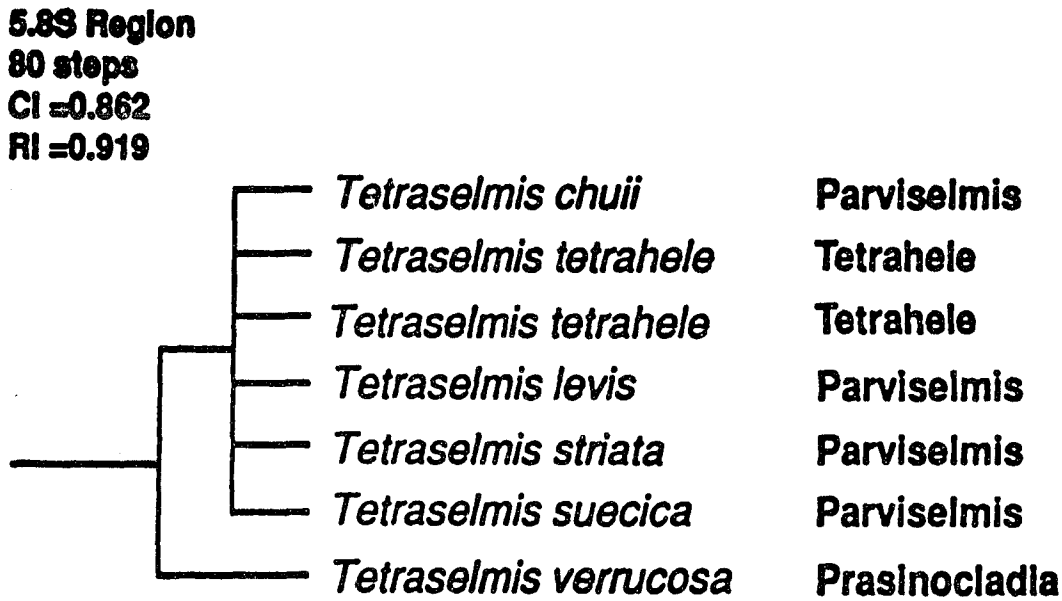

Figure 4.1. The single parsimonious cladogram from analysis of the 5.8S rDNA.

subgenera, which both have cytoplasmic channels which traverse the pyrenoid matrix.

The analysis of the full alignment (Appendix B), which includes portions of the ITS1 and ITS2 regions in addition to the 5.8S sequence, resulted in a single cladogram (length = 355, CI = 0.885, and RI = 0.876) (Fig. 4.2). With the full alignment relationships within the Tetrathele + Parviselmis clade can be distinguished. Neither subgenus is monophyletic. Three of the Parviselmis species, T. striata, T. levis, and T. suecica, emerge from an unresolved node. The forth Parviselmis species T. chuii is sister to the Tetrathele species T. tetrahele (UW421). The primary differences used to distinguish the two subgenera involve the size of the pyrenoid (Hori et al. 1982). The non-monophyly of the two subgenera indicates that an increase or decrease in the size of the pyrenoid may have occurred several times during the evolution of Tetraselmis.

The site of collection of each Tetraselmis strain is provided in Hori et al. (1982), Hori et al. (1983), and Hori et al. (1986) (Fig. 4.2).

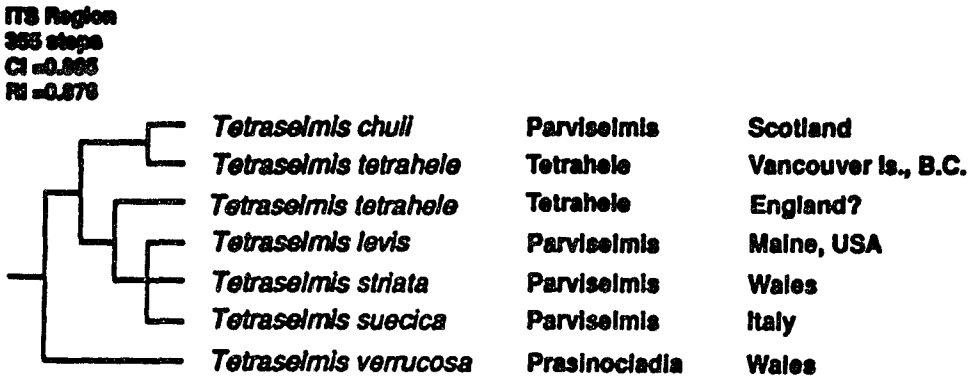

Figure 4.2. The single most parsimonious cladogram generated from the ITS1, 5.8S rDNA, and ITS2 sequences.

Tetraselmis suecica was collected off the coast of Italy, and T. levis and T. striata are from the North Atlantic. The sister taxa T. chuii, and T. tetrahele (UW421) are from the Atlantic and Pacific oceans, respectively. The biogeographic information does not reveal any clear-cut patterns. However, the small sampling of taxa may be obscuring biogeographic patterns. No Asian strains were examined, and only taxa from the northern hemisphere were included. With addition of more taxa from Asia and the southern hemisphere biogeographic patterns may become more evident.

In this preliminary study of sequence variation in Tetraselmis, some patterns are becoming evident. In contrast to Chlamydomonas, Tetraselmis shows little variation in the LSU or SSU sequences, even in the most variable regions (Kantz et al. 1990). The 5.8S sequence shows enough variation to distinguish only one of the subgenera from the other two. Variation within the ITS regions is great enough to distinguish the three subgenera.

Why Tetraselmis shows less variability than Chlamydomonas or Pandorina is not known. It could be

that the genus itself has arisen quite recently and has not had time to diverge significantly; this explanation is counter to the view, based on the predominance of putatively primitive ultrastructural characters in Tetraselmis, that the genus is ancient (Norris 1980). Alternatively, some phenomenon may be slowing the rate of divergence among the lineages. Tetraselmis has exclusively asexual reproduction; Chlamydomonas and Pandorina have a sexual lifecycle. Perhaps the sexual lifecycle is somehow accelerating the divergence of the various lineages through recombination. Clearly, additional molecular studies of green algal flagellates with different lifecycles is necessary. Similarly, comparisons between green algal flagellates and macroscopic green algae might reveal different levels of intrageneric sequence variation that ultimately might be correlated with life cycle differences or molecular mechanisms that affect the rate of molecular divergence.

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CONCLUSION

Cladistic analysis of the ultrastructural features of the flagellar apparatus, cell covering, mitosis and cytokinesis, and some biochemical characters resulted in a monophyletic Pleurastrophyceae and a monophyletic Prasinophyceae. These results are congruent, in many respects, to current classifications of these algal classes. However, the results are not congruent with those of the ribosomal RNA sequence analysis.

The cladograms generated from the ribosomal RNA gene sequence data show that the Pleurastrophyceae is not monophyletic, though the pleurastrophycean taxa are more closely related to the Chlorophyceae than to any other class. Nor is the Prasinophyceae a monophyletic group -- some prasinophycean taxa are more closely allied to the other classes of green algae than with each other. These results are robust under different models of evolution, and under different methods of analysis (maximum likelihood and Evolutionary Parsimony). Furthermore, the greater

frequency of transitional compared to transversional changes did not result in a high level of randomness in the data. Indeed, randomization tests of the sequence data show a high level of phylogenetic signal and a low level of randomness in the data.

By including molecular, ultrastructural, and biochemical data, this study is the most exhaustive phylogenetic analysis to date of the Prasinophyceae and Pleurastrophyceae. However, the incongruities between the different data sets underscores the need for additional systematic studies, both molecular and ultrastructural, of the major lineages of green algae. The preponderance of characters for the flagellar apparatus and cell covering, in the ultrastructural data set, may be biasing the cladistic analysis if there is non-independence of characters in these character systems. Ultrastructural studies of additional evolutionarily conserved character systems, such as mitosis and cytokinesis, will provide independent characters to test hypotheses of green algal evolution.

Furthermore, the cladogram of the rRNA gene sequence analysis is, in essence, a hypothesis of

evolution that is based on a single gene. If there are structural and functional correlations of characters within the sequence data, then, as in the ultrastructural data, the analysis may be biased. Additional studies of independent molecular data sets of conserved nuclear, chloroplast, and mitochondrial genes will provide much needed information about relationships among the lineages of green algae.

APPENDIX A

ALIGNED RIBOSOMAL RNA SEQUENCES

Appendix A. Aligned partial sequences of the SSU (primers 18E, 18G, 18H, 18J, and 18L) and LSU (primers 26C, and 26D) rRNA for each taxon included in the phylogenetic analysis. Highly variable regions which were excluded from the analysis are denoted by a solid line above the alignment.

```
18E Primer
[                    1                                                  50]
Glycine              .......... .......... ......GGTA ...TCT.ACT .ACTCGG.AT
Zamia                ATTAAATCAG TTATAGTTTC TTTGATGGTA ..CTCT.GCT .ACACGG.AT
Equisetum            ATTAAATCAG TTATAGTTTC TTTGATGGTA ..CCTT.GCT .ACTCGG.AT
Klebsormidium        .......... .......... .......... ..CCTT..AT .ACTCGG.AT
Bryopsis             XATAATCCAG TGATXATCTC GTCGGCGTGX .......ACG .TCTCGG.AT
Enteromorpha         ATTAAATCAG TTAGAGTTTA TTTGATGGTA ..CCAC.ACT .ACTCGG.AT
Chlamydomonas.eug    ATTAAATCAG TTATAATTTA TTTXATGGTA ..CT.T.ACT .ACTTGG.AT
Chlamydomonas.moe    ATTAAATCAG TTATAATTTA TTTGATGGTA ..CT.T.ACT .ACTTGG.AT
Chlamydomonas.rei    .......... .......... .....TGGTA ..CC.T.XCT .ACTCGG.AT
Asteromonas          .......... .......... .......... .......... ..........
Chlorella            AUUAAAUCAG UUAUAGUUUA UUUGAUGGUA ..CU.U.ACU .ACUCGG.AU
Nanochlorum          AUUAAAUCAG uuAUagUUua uuUGAUGGUA ..CC.U.ACU uACuCGg.AU
Tetraselmis.carter   .......... ..ATAGTTTA TTTGATGGTA ..CC.T.ACT .ACTCGG.AT
Tetraselmis.levis    .......... .......... .......... .......... .....GG.AT
Pseudotrebouxia      .......... .......... TTTGATGGTR G.CCTT.ACT .RCTCGG.AT
Pleurastrum          ......TCAG TTATAGTTTA TTTGATGGTA ..CACT.ACT .ACTCGG.AT
Friedmannia          ATTAAATCAG TTATAGTTTA TTTGATGGTA ....CC.CTT .ACTCGG.AT
Microthamnion        .TTAAATCAG TTATAGTTTA TTTGATGGTA ..CCTT.ACT .ACTCGG.AT
Myrmecia             .......... .......... .......... ........TT .ACTCGG.AT
Chlorosarcina        .......... .......... .......... .......... ..........
Pyramimonas.parkae   .......... .......... .......... .......... ..........
Pyramimonas.virgini  .......... .......... .......... .......... ..........
Mesostigma           ATTAAATCAG TTATAGTTTA TTTGATGGTA ..CCCT.ACT .ACTCGG.AT
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    .........G TTATAGTTTA TTTGATGGTA ..CCTT.ACT .ACTCGG.AT
Micromonas           .......... ..ATXGTTTC TTTGGTGGTG ..TTTT.ACT .ACATGG.AT
Mantoniella          .......... .......... .......... .......... ..........
Pseudoscourfieldia   ATTAAATCAG TTATAGTK.A TTTGATGGTA ..CCTT.ACT GACTCGG.AT
Nephroselmis.pyrifo  .......... .......... .......... ..CCTT.RCT .RCTCGG.AT
Pedinomonas.minutis  .........G AAATXATTTC TTTGATGGTG ..AAAA.TCT .ACACGG.AT
Costaria             ATTATATCAG TCATAGTTTA TTTGAAAGTC ..CCTT.ACT .ACATGG.AT
Saccharomyces        ATTAAATCAG TTATCGTTTA TTTGATAGTT ..CCTTTACT .ACATGGTAT
Phaeodactylum        .......... .......... .......... .......... ..........

[                    51                                                 100]
Glycine              AACCGTAGTA A.TTCTAGAG CTAATACGTG C.AACAAACC CCGACTTCT.
Zamia                AACCGTAGTA A.TTCTAGAG CTAATACGTG C.ACCAAATC CCGACTTTT.
Equisetum            AACCGTAGTA A.TTCTAGAG CTAATACGTG C.ACCAACTC CCGACTTCT.
Klebsormidium        AACCGTAGTA AGTTCTA.AG CTAATACGTG C.ACCAAATC CCGACTTCT.
Bryopsis             ACCCGCGGXX X.TTCTGTGG ATAATCGCGT T.CTCXXCGA GCAGGCACT.
Enteromorpha         AACCGTAGTA A.AGCTACAG CTAATACGTG C.GTA.ACTC CCGACYCNC.
Chlamydomonas.eug    AACCGTAGTA A.TTCTAGAG CTAATACATG C.GGATRATC CCAACTTCT.
Chlamydomonas.moe    AACCGTAGTA A.TTCTAGAG CTAATACATG C.GGATAATC CCAACTTCT.
Chlamydomonas.rei    AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GCACAA.C CCGACTTCT.
Asteromonas          .......... .......... .......... .......... ..........
Chlorella            ACCCGUAGUA A.AUCUAGAG CUAAUACGUG C.GUA.AAUC CCGACUUCU.
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Nanochlorum              AcCcgUAGUA A.UUCUAgaG cUaAUaCGuG C.GCA.CAUC CCgaCUUCU.
Tetraselmis.carter       AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GTA.AATC CCGACTTCT.
Tetraselmis.levis        AACCGTAGTX A.TTCTXXAG CTAATACGTG C.GTA.AATC CCGACKTCT.
Pseudotrebouxia          AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GCA.CATC CCGACTCRC.
Pleurastrum              AACCGTAGTA A.TTCTAGGG CTAATACGTG C.GTA.AATC CCGACTTCT.
Friedmannia              AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GTA.AACC CCGACTTCT.
Microthamnion            AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GTA.AATC CCGACTCXC.
Myrmecia                 AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GTA.AACC CCGACTTTT.
Chlorosarcina            .......... .......... .......... .......... ..........
Pyramimonas.parkae       .......... ..TTCTAGAG CTAATACGTG C.GCA.ACTC CCGACTTCT.
Pyramimonas.virgini      .......... .......... .......... .......... ..........
Mesostigma               ACCCGTAGTA A.TTCTAGAG CTAATACGTG C.ACCAAGTC CCGACTCTC.
Pedinomonas.tubercu      .......... .......... .......... .......... ..........
Pedinomonas.minor        AACCGTAGTA A.CCCTAGAG CTAATACGTG C.GCX.XATC CCGACTCTT.
Micromonas               AACCGTAGTA A.TTCTXGXG CTAATACATG C.GTA.AATC CCGACTTC..
Mantoniella              .......... .......... ..AATACATG C.GTR.AATC CCGACKTC..
Pseudoscourfieldia       AACCGTAGTA A.TTCTAGAG CTAATACGTG C.GCA.ACAC CCGACTTC..
Nephroselmis.pyrifo      XACCGTAGTA R.KTCTRGRG CTAATACGTG C.GCA.ACAC CCGACTTC..
Pedinomonas.minutis      ACCCGTXGTX A.TTCTXGAG CTAATACGTS C.GTX.AA.. CTCCATT...
Costaria                 AACCGTAGTA A.TTCTAGAG CTAATACATG CATGCAAGGC CCAACTGCTT
Saccharomyces            AACCGTGGTA A.TTCTAGAG CTAATACATG C.TTAAAATC TCGACCCTTT
Phaeodactylum            .......... .......... .......... .......... ........SS

[                        101                                                150]
Glycine                  GGAA.GGGAT GC.ATTTATT AGATAAAAGG TCA.AC.... ACAGGCT.CT
Zamia                    TGAA.GGGAC GC.ATCTATT AGATAAAAGG CCG.M..... GCGGGCT.TT
Equisetum                .GGR.GGGAX GC.ATTTATT AGATAAAAGG CCG.AT.... GCGGGCT.GT
Klebsormidium            GGAA.GGGAC GTGATTTATT AGATAAAAGG CCA.AT.... GCGGGCT..T
Bryopsis                 CGTT.GTCAG AT.GTAAGCX ATCCTXGTXG AAG.GC.... ..........
Enteromorpha             .GAA.GGGAC GT.MTTTATT AGATTCMAGA CCG.AC.... .CGTGCT..T
Chlamydomonas.eug        GGAA.GGGAC GT.ATTTATT AGATAAAAGG CCA.GC.... .CGTGCT..T
Chlamydomonas.moe        GGAA.GGGAC GT.ATTTATT AGATAAAAGG CCA.GC.... .CGTGCT..T
Chlamydomonas.rei        GGAA.GGGTC GT.ATTTATT AGATAAAAGG CCA.GC.... ....GCT.CT
Asteromonas              .......... .......... .......... .......... ..........
Chlorella                GGAA.GGGAC GU.AUUUAUU AGAUAAAAGG CCG.AC.... .CGGGCUUCU
Nanochlorum              GGaa.GGGAc GU.AUUUAUU AgAUaAAAgg ccg.ac.... .CGGAUU..G
Tetraselmis.carter       GGAA.GGGAC GT.ATTTATT AGATTTAAXG SCG.RG.... .CSAGCT.TT
Tetraselmis.levis        GGAA.GGGAS GT.ATTTATT AGATTTMAGG SCG.GA.... .GCXXCT.TT
Pseudotrebouxia          .GAA.GGGAS GK.RTTTATT AGATAAAAGG SCG.AGCC.. .GGGGCR..R
Pleurastrum              GGAA.GGGAC GT.ATTTATT AGATAAAAGG CCG.AC.... .CGGACT.CK
Friedmannia              GGAA.GGGGX XT.ATTTATT AGATAAAAGG XXG.AC.... .GGGCTTG..
Microthamnion            .GAA.GGGAC GT.ATTTATT AGATAAAAGG CCG.AC.... ....GCT.TT
Myrmecia                 GGAA.GGGXX XT.ATTTATT AGATAAAAGA CGA.C..... .CGGCTT.GC
Chlorosarcina            .......... .......... .......... .......... ..........
Pyramimonas.parkae       GGAA..GGAC GT.ATTTATT AGATAAAAAG ACC.AG.... .GCCCTC.GG
Pyramimonas.virgini      .......... .......... .......... .......... ..........
Mesostigma               GGAA.GGGAY GT.ATTTATT AGATCCAAGA CCA.ATA... .CGGCTT.CG
Pedinomonas.tubercu      .......... .......... .......... .......... ..........
Pedinomonas.minor        GGAA.GGGAC GT.ATTTATT AGATAAAAGG CCA.GC.... .CGAGCT.TG
Micromonas               GGAA.GGGAC GT.ATTTATT AGAT.AAAGA CSS.AC.... ..........
Mantoniella              GGRR.GGGRS GT.RTTTATT AGAT.XAAGA CCX.AC.... ..........
Pseudoscourfieldia       GSAXAGGGTK GX.XTATATT ACATAAAAGA CCG.AC.... ....GCT.TC
Nephroselmis.pyrifo      GGAA.GGGTT GT.RTATATT AGATAAAAGA CCG.AC.... ....SCT.TC
Pedinomonas.minutis      ......GGAG GT.XTTTXTT XGRT.CCXAA CCA.GC.... .....CT.TX
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Costaria                CGGCGGACGG GCTGCATTGA TTAGACCGAA ACCAATGCG. ..........
Saccharomyces           GGAA.GAGAT GT.ATTTATT AGATAAAAAA TCA.AT.... ...GTCT..T
Phaeodactylum           KKCCGGGGKA GK.ATTTATT AGAT.TGAAA CCA.M..... ...CTCT..C

[                       151                                                200]
Glycine                 G.CCTGT... .TGCTTTGAT GATTCATGAT AACTCGTC.. GGATCGCA.C
Zamia                   G.CCCGG... .TCGTTTGGT GAATCATGAT ACCTTGAT.. GGATTGCA.T
Equisetum               G.CCCGG... .TAACGXXGK KATTCXXGAT AACTTCCC.. GGATCGCA.C
Klebsormidium           ..CCCGG... .TATTGCGGT GAATCATGAT AACTCGTCX. GAATCGCA.C
Bryopsis                .......... ........GA ACCXXXGASS ATCGATGTC. XAATGCCCA.
Enteromorpha            G.CMCGT... ...CTTTGGT GAATCATGGT AACTTCAC.. GAATCGCA.S
Chlamydomonas.eug       G.CACGA... ..TCCTGGTT GATTCATGAT AACTTCAC.. GAATCGCA.T
Chlamydomonas.moe       G.CACGA... ..TCCTGGTT GATTCATGAT AACTTCAC.. GAATCGCA.T
Chlamydomonas.rei       G.CCCGA... ....CXXXXX AWXXXATGAT AACTTCAC.. GAATCGTA.T
Asteromonas             .......... .......... .......... .......... ..........
Chlorella               G.CCCGA... ..CUCGCGGU GAAUCAUGAU AACUUCAC.. GAAUCGCA.U
Nanochlorum             U.UCCGa... ..cuCgcGGU gacucaUGAU AACUUCAC.. GAAUCGCa.U
Tetraselmis.carter      X.CKSGT... ..CKKXCGGT GAAKCAGKAT AACTTCAC.. GAATCGCA.T
Tetraselmis.levis       G.CTCGT... ..CTTGCGGT GAATCSTGAT AACTTCXC.. GAATCXCA.T
Pseudotrebouxia         ..CCCGASSS GASKCGCGGT GAATCAKGAT AACKKCAC.. GAATCGTA.S
Pleurastrum             G.TCCGA... ..CCCGCGGT GAACCRTGAT AACTTCAC.. GAATCGCA.T
Friedmannia             ..CCCGA... ..CTCGCGGT GAATCATGAT AACTTCAC.. GAATCGCA.T
Microthamnion           G.CXCGA... ...CTGCGGT GAATCATGAT AACTTCAC.. GAATCGCA.T
Myrmecia                ...CCGA... ..CTCGCGGT GAATCATGAT AACTTCAC.. GAATCGCA.T
Chlorosarcina           .......... .......... .......... .......... ..........
Pyramimonas.parkae      G.CGT..... ..TTTGTGGT GAATCATGAT AACTTGTC.. GGATCGCA.T
Pyramimonas.virgini     .......... .......... .......... .......... ..........
Mesostigma              G.CCGGC... ..ATTGCGGT GAATCATAAT AACTCGTC.. GAATCGCA.T
Pedinomonas.tubercu     .......... .......... .......... .......... ..........
Pedinomonas.minor       C.GKCGA... ..CCTGCGGT GAATTCATGG ATAACTTCAC GAATCGCA.C
Micromonas              ..CTCGT... ..TCTGCGGT GAATCATGAT AACTTCXC.. GGATCGCA.T
Mantoniella             ..CTCGT... ..TCTGCGGT GAATCAKGAT XACTTSXC.. GGATCXCA.T
Pseudoscourfieldia      G..GCGT... ..TCTTCGGT GAATCMKGAT ATTTCCMC.. GGATCGMACT
Nephroselmis.pyrifo     G..GCGT... ..TCTTCGGT GAATCATGAT ATTTCCAC.. GGATCGCA.T
Pedinomonas.minutis     X.GGGGT... ..TTTCTGXT GAATCXTGAT XACTXTTC.. ..........
Costaria                .....TCTTC GGAGGTTTTT GAATCATAAT CACTTGCG.. .GATCGCA..
Saccharomyces           ..CGGAC... ..TCTTTGAT GATTCATAAT AACTTTTC.. GAATCGCA.T
Phaeodactylum           ..GGGGT... ..GRTGKGGT GATTCATAAT AAGCYTCG.. GAAY.GCA.T

[                       201        214]
Glycine                 GGCCTTTGTG CCGG
Zamia                   GGCCCTCGAG CCGG
Equisetum               GGCCTTTGSK CCGG
Klebsormidium           GGCCTTTGCG CTGX
Bryopsis                .......... ....
Enteromorpha            GG..TTTACC CCGG
Chlamydomonas.eug       GGCCTT.... ....
Chlamydomonas.moe       GGCCTTGTGC CGGC
Chlamydomonas.rei       GGGCTCGTCC CGAC
Asteromonas             .......... ....
Chlorella               GGCCUUGUGC CGGC
Nanochlorum             GGCCUCGUGC CGGC
Tetraselmis.carter      GGCCTCCGCG CCGG
Tetraselmis.levis       .......... ....
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Pseudotrebouxia       G.CCTTGTGC CGXC
Pleurastrum           GGCCTTGCXC C...
Friedmannia           GGCCTTGTGC CGGC
Microthamnion         GGCCTTGCGC CGG.
Myrmecia              GGCCTTGTGC CGG.
Chlorosarcina         .......... ....
Pyramimonas.parkae    GGCCTTGTGC CGGC
Pyramimonas.virgini   .......... ....
Mesostigma            GGCCTCCGCG CCG.
Pedinomonas.tubercu   .......... ....
Pedinomonas.minor     GGCCTTGTGC CGGC
Micromonas            GG.CTT..CA A...
Mantoniella           GG.CTT..CA AGCC
Pseudoscourfieldia    GGGCKK..CC CC..
Nephroselmis.pyrifo   XX.CTT..CC C...
Pedinomonas.minutis   .......... ....
Costaria              .CGCTTCGGC GGCG
Saccharomyces         GGCCTTGTGC TGGC
Phaeodactylum         GCYTTT..GC CGGC

18G Primer
[                     215                                                  264]
Glycine               CGAC.GCATC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Zamia                 CGAC.GCTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGCAGGATAG
Equisetum             .......... .......... ...CCCTATC AACTTTCGAT GGTAGGAKAG
Klebsormidium         .......... .......... .......... .......... ..........
Bryopsis              .......... .......... .......... .......... ..........
Enteromorpha          .......... .......... .......... .......... ..........
Chlamydomonas.eug     .......... .......... .......... .......... ..........
Chlamydomonas.moe     .......... .......... .......... .......... ..........
Chlamydomonas.rei     ..........T TTKCCCTATC AACTTTCGAT GGTAGGATAG
Asteromonas           CGAT.GTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Chlorella             CGAU.GUUUC AUUCAAAUUU CUGCCCUAUC AACUUUUGAU GGUAGGAUAG
Nanochlorum           CGAU.GUUUC AuucAAAUuu CUGCCCUaUc aACUuuugAU GGUAGGAUAG
Tetraselmis.carter    CGAT.GTTTC ATTCAAATTT CTGCCCTATC AATTTGCGAT GGTAGGATAG
Tetraselmis.levis     .......... .......... .......... .......... ..........
Pseudotrebouxia       .......... .......... .......... .......... ..........
Pleurastrum           .......... .......... .......... .......... ..........
Friedmannia           CGATAGTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTTGGATAG
Microthamnion         CGAT.GTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Myrmecia              CGAATGTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTTGGATAG
Chlorosarcina         .......... .......... .......... .......... ..........
Pyramimonas.parkae    CGAC.GTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Pyramimonas.virgini   CGAC.GTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Mesostigma            GCGATGTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Pedinomonas.tubercu   GCGATGTTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
Pedinomonas.minor     .......... .......... .......... .......... ..........
Micromonas            .......... .......... .......... .......... ..........
Mantoniella           GCGATGTTCC ATTCAAATTT CTGCCCTATC AACTTTCGAC GGTAGGATAG
Pseudoscourfieldia    .......... .......... .......... .......... ..........
Nephroselmis.pyrifo   .......... .......... .......... .......... ..........
Pedinomonas.minutis   .......... .......... .......... .......... ..........
Costaria              GCGACGTTTC ATTCAAGTTT CTGCCCTATC AGCTTTGGAT GGTAGGGTAT
Saccharomyces         GCGATGGTTC ATTCAAATTT CTGCCCTATC AACTTTCGAT GGTAGGATAG
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Phaeodactylum      .......... .......... .......... .......... ..........

[                  265                                                 314]
Glycine            TGGCCTACCA TGGTGGTGAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Zamia              AGGCCTACCA TGGTGGTGAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Equisetum          AGGCCTACCA TGGTGGTGAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Klebsormidium      .......... ......TAAC GGGTGACGGA GAATTXGGG. TTCGATTCCG
Bryopsis           .......... .......... ........GG AAATCAGGG. TTTGATTCCG
Enteromorpha       .......... .......... .......... ..ATTAGGGG TTCGATTCCG
Chlamydomonas.eug  .......... .....TXACG GGTGACGGAX XATXCAGGG. TTCGATTCCG
Chlamydomonas.moe  .......... .......XAC GGCTGACXXA XXATCAGGG. TTCGATTCCG
Chlamydomonas.rei  AGGCCTACCA TGGTGGTAAC GGGTGACGGA GGATTAGGG. TTCGATTCCG
Asteromonas        AGGCCTACCA TGGTGGTAAC GGGTGACGGG GGATTAGGG. TTCGATTCCG
Chlorella          AGGCCUACCA UGGUGGUAAC GGGUGACGGA GGAUUAGGG. UUCGAUUCCG
Nanochlorum        AGGCCUACCA UGGUgguaAc GGGUGACGGA GAAUUAGGG. UUCgauUCCG
Tetraselmis.carter AGGCCTACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Tetraselmis.levis  .......... .......... .......... .......... ..........
Pseudotrebouxia    .......... .......... .......... GGATTAGGG. TTCGXTTCCG
Pleurastrum        .......... .......... .......... ..ATTAGGG. TTCGATTCCG
Friedmannia        AGGCCAACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Microthamnion      AGGCCTACCA TGGTGGTAAC GGGTGACGGA GGATTAGGG. TTCGATTCCG
Myrmecia           AGGCCAACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Chlorosarcina      .......... .......... .......... .......... ..........
Pyramimonas.parkae AGGCCTACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Pyramimonas.virgini AGGCCTACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Mesostigma         AGGCCTACCA TGGTGGTAAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Pedinomonas.tubercu AGGCCTACCA TGGTGGTA.. .....ACGGA GGATTAGGG. TTCGATTCCG
Pedinomonas.minor  .......... .......... .......... .......... ..........
Micromonas         .......... .......... .......... .......... ..........
Mantoniella        AGGCCTACCG TGGTGTTCAC GGGTGACGGA GAATTAGGG. TTCGATTCCG
Pseudoscourfieldia .......... .......... .......... .......... ..........
Nephroselmis.pyrifo .......... .......... .......... .......... ..........
Pedinomonas.minutis .......... .......... CGGTGACGX. GAATTXGGG. TTCGXTTCCG
Costaria           TGGCCTACCA TGGCTTTAAC GGGTAACGGG GAATTGGGG. TTCGATTCCG
Saccharomyces      TGGCCTACCA TGGTTTCAAC GGGTAACGGG GAATAAGGG. TTCGATTCCG
Phaeodactylum      .......... .......... .......... ........G. TT.G.TTCCG

[                  315                                                 364]
Glycine            GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Zamia              GA..GAGGG. AGCCCGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Equisetum          GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCG
Klebsormidium      GA..GAXGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Bryopsis           GA..GAKGG. AGCCTGAGA. XXCGGCTACC A.SATCCAXG GAAGGCAGCA
Enteromorpha       GAGGGAGGG. AGCCT.AGG. XXCGGCTACC AGCATCCGAG GAAGGCAGCX
Chlamydomonas.eug  GA..GAGGG. AGCCTGAGX. XXCGGCTACC A.CATCCAAG GGAGGCAGCA
Chlamydomonas.moe  GR..GAGGG. AGCCTKAGX. XXCGGCTRCC A.SATCCXXG GAAGGCXGCX
Chlamydomonas.rei  GA..GAGGG. AGCCTGAGA. GATGGCTACC A.CATCCAAG GAAGGCAGCA
Asteromonas        GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Chlorella          GA..GAGGG. AGCCUGAGA. AACGGCUACC A.CAUCCAAG GAAGGCAGCA
Nanochlorum        XG..AGAGGg AGCCUGAGA. AACGGCUaCC A.CaUCCAAG GAaGGCAGCA
Tetraselmis.carter GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Tetraselmis.levis  .......... .......... .......... .......... ..........
Pseudotrebouxia    GA..GAGGG. AGCCTGAGX. XXCGGCTXCC A.CATCSXXG GAAGGCAGCX
Pleurastrum        GX..GAGGG. AGCCTGAGX. XXCGGCTACC A.CATCCXAG GAAGGCAGCX
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Friedmannia              GA..GAGGG..AGCCTGAGA. GACGGCTACC A.CATCCAAG GAAGGCAGCA
Microthamnion            GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Myrmecia                 GA..GAGGG. AGCCTGAGA. GACGGCTACC A.CATCCAAG GAAGGCAGCA
Chlorosarcina            .......... .......... .......... .......... ..........
Pyramimonas.parkae       GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Pyramimonas.virgini      GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Mesostigma               GA..GAGGG. AGCCTGAGA. AACGGCTACC T.CATCCAAG GAAGGCAGCA
Pedinomonas.tubercu      GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Pedinomonas.minor        .......... .......... .......... .......... ..........
Micromonas               .......... .......... .......... .......... ..........
Mantoniella              GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Pseudoscourfieldia       .......... .......... .......... .......... ..........
Nephroselmis.pyrifo      .......... .......... ..CGGCTACC A.CATCCAAK KAAGGCRGCX
Pedinomonas.minutis      GX..GAGGG. AGCCTGAGA. AACGGCTACC X.CATCXAAG GAAGGCXGCG
Costaria                 GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGG.AGCA
Saccharomyces            GA..GAGGG. AGCCTGAGA. AACGGCTACC A.CATCCAAG GAAGGCAGCA
Phaeodactylum            GA..XAGGG. AGCCTAGAGX GACCGGTACC A.C.TCCXAG GAAGGCA..A

[                        365                                                414]
Glycine                  GGCGCGCAAA TTACCCAATC CTGAC.ACG. GGG..AGG.. ...TAGT.GA
Zamia                    GGCGCGCAAA TTACCCAATC CTGAC.ACG. GGG..AGG.. ...TAGT.GA
Equisetum                GGCGCGCAAA TTACCCAATC C.GAC.ACG. GGG..AGG.. ...TAGT.GA
Klebsormidium            GGCGCGCXXA TTXCCCAATC CTGXT.XCA. GGG..AGG.. ...TAGT.GA
Bryopsis                 .GCGCGCAAA TTXCCCAATC CCGAA..... GGGTGAGGTA GGGTAGT.GA
Enteromorpha             GGCGCGCAAA TTACCCAATC CTGXXG.CA. GGG..AGG.. ...TAGT.GA
Chlamydomonas.eug        GGCGCGTXAA TTACCCAATC C.GAGTACG. GGG..AGG.. ...TAGT.GA
Chlamydomonas.moe        GXCGCGTXAA TTACCCAATC CXXX..XXG. GGG..AGG.. ...TX.T.GA
Chlamydomonas.rei        GGCGCGCXXA TTACCCAATX CCGAX.ACG. GGG..AGG.. ...TAGT.GA
Asteromonas              GGCGCGCAAA TTACCCAATC CCAAC.ACG. GGG..AGG.. ...TAGT.GA
Chlorella                GGCGCGCAAA UUACCCAAUC CUGAC.ACA. GGG..AGG.. ...UAGU.GA
Nanochlorum              GGCGCGCAAA UUACCcaaUC CUGAC.ACA. GGG..aGG.. ...UAGU.ga
Tetraselmis.carter       GGCGCGCAAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Tetraselmis.levis        .......... .......... .......... .......... ..........
Pseudotrebouxia          XGCGCSCXXA TTACCCAATC CTGXT.XCA. GGG..AGG.. ...TAXT.GA
Pleurastrum              SGCGCGCXXA TTACCCAATC CTGAS.XXG. GGG..AGG.. ...TAGT.GA
Friedmannia              GGCGXGCAAA TTACCCAATC CTGAC.ACA. GGG..AXG.. ...TAGT.GA
Microthamnion            GGCGCGCAAA TTACCCAATC C.GAC.ACG. GGG..AGG.. ...TAGT.GA
Myrmecia                 GG........ .......... .......... .......... ..........
Chlorosarcina            .......... .......... .......... .......... ..........
Pyramimonas.parkae       GGXGX..AAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Pyramimonas.virgini      GGCGCGCAAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Mesostigma               GGCGCGCAAA TTACCCAATC CTGAT.ACA. GGG..AGG.. ...TAGT.GA
Pedinomonas.tubercu      GGCGCGCAAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Pedinomonas.minor        .......... .......... .......... .......... ..........
Micromonas               .......... .......... .......... .......... ..........
Mantoniella              GGCGCGCAAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Pseudoscourfieldia       .......... .......... .......... .......... ..........
Nephroselmis.pyrifo      KGCGCGCXXA TTASCCAATC CTGAC.XCA. GGG..AGG.. ...TAGT.GA
Pedinomonas.minutis      GGCGCGCXXA TTACCCAATC CGAGX.AXXX GGG..AGG.. ...XAGTGGA
Costaria                 GGCGCGTAAA TTACCCAATC CTGAC.ACA. GGG..AGG.. ...TAGT.GA
Saccharomyces            GGCGCGCAAA TTACCCAATC CTAAT.TCA. GGG..AGG.. ...TAGT.GA
Phaeodactylum            GGCGCXXAAA TTACCCAATC CTGAC.XCA. GGG..AGX.. ...TXGX.GA

[                        415                                                464]
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Glycine                 CAATAAATAA CAATA.CC.G GGCTCA.TTG A.G.TCTGGT AATTGGAATG
Zamia                   CAATAAATAA CAATA.CT.G GGCTCA.TCG A.G.TCTGGT AATTGGAATG
Equisetum               CAATAAATAA CAATA.CT.G GGCTTTTACA A.G.TCTGGT AATTGGAATG
Klebsormidium           CXATAAATAA CAATG.CT.G GGCTTTTCAA A.G.TCTGGC AATTGGAATG
Bryopsis                CXAGACGTAA CAACG.TC.G TGCTCATAGA G.T.GGGACG A.TTGGAXXX
Enteromorpha            CAATAAATAT CAXT..TCTG GGC.CACTTG G...TCCGGT AATTGGAATG
Chlamydomonas.eug       CAATAAATAA CAATA.TC.G GGCATC.CA. ATG.TCTGAT AATTGGAAT.
Chlamydomonas.moe       CAATAAATAA CAATA.TC.G GGCATC.CA. ATG.TCTGAT AATTGGAATG
Chlamydomonas.rei       CAATAAATAA CAATA.CC.G GSXXCGC... ..G.TCTGGX AATTGGAATG
Asteromonas             CAATAAATAA CAATACCG.G GCATTCTT.. ..G.TCTGGT AATTGGAATG
Chlorella               CAAUAAAUAA CAAUACUG.G GCCUUUUCAG ..G.UCUGGU AAUUGGAAUG
Nanochlorum             caAuAAAUAA CAAUACCG.G GCCUUUG... ..G.UCUGGU AaUUGGAAUG
Tetraselmis.carter      CAATAAATAA CAATACCG.G GCTTTT.CAA .......... ..........
Tetraselmis.levis       .......... .......... .......... .......... ..........
Pseudotrebouxia         CAATAAATAA CAATACCG.G GCTTTTTCAA ..G.TCTGGT AATTGGXATX
Pleurastrum             CAATAAATAA CAATACCG.G GCATTTA.AT ..G.TCTGGT AATTGGAATG
Friedmannia             CAATAAATAA CAATACCG.G GCTTTTTCAA ..G.TCTGGT AA........
Microthamnion           CAATAAATAA CAATACCG.G GCATTTT.AT ..G.TCTGGT AATTGGAATG
Myrmecia                .......... .......... .......... .......... ..........
Chlorosarcina           .......... .......... .......... .......... ..........
Pyramimonas.parkae      C......... .......... .......... .......... ..........
Pyramimonas.virgini     CAATAAATAA CAATACCG.G GCTTTTTCAA ..G..... ..........
Mesostigma              CAATAAATAA CAATATCG.G GCTCTT.CGA ..G.TCTGAT ..........
Pedinomonas.tubercu     CAATAAATAA CAATGCCG.G GCTTTT.CAA ..G.TCT... ..........
Pedinomonas.minor       .......... .......... .......... .......... ..........
Micromonas              .......... .......... .......... .......... ..........
Mantoniella             CAATAAATAA CAATATCG.G GGTTTTTCAA .......... ..........
Pseudoscourfieldia      .......... .......... .......... .......... ..........
Nephroselmis.pyrifo     CXATAAATAA CAATACCG.G GCTTTTTCAA ..G.TCTGGT AATTGGAATG
Pedinomonas.minutis     CXAGAAATAT CGTGACTG.T GCCCTX.... ....TGTGGC AGAGTCTTCG
Costaria                CAATAAATAA CAATGCCG.G GTTATA.CAA ..G.TCTGGC AATTGGAATG
Saccharomyces           CAATAAATAA CGATACAG.G GCCCATTCG. ..GGTCTTGT AATTGGAATG
Phaeodactylum           CAATAAATAC CAXXXCCG.G GCCTTTCXA. ..GGTCTGGC TTXTG.AATG

18H Primer
[                       465                                                514]
Glycine                 .......... .......... .......... .......... ..........
Zamia                   TCGGGACCGG AGTAATGATT AACAGGGACG GTCGGGGGCA TTCGTATTTC
Equisetum               .......... .......... .......... .......... ..........
Klebsormidium           .......... .......... .......... .......... ..........
Bryopsis                .......... .......... .......... .......... ..........
Enteromorpha            .......... .......... .......... .......... ..........
Chlamydomonas.eug       .......... .......... .......... .......... ..GGTATTTC
Chlamydomonas.moe       .......... .......... .......... .......... ..........
Chlamydomonas.rei       .......... .......... .......... .......... ..........
Asteromonas             .......... .......... .......... .......... ..........
Chlorella               GUAGGACCGG AGUAAUGAUU AAGAGGGACA GUCGGGGGCA UUCGUAUUUC
Nanochlorum             GUAGGACCGG AGUAAUGAUU AAGAGGGACA gUCgggGGCA UUCGUaUUUC
Tetraselmis.carter      .......... .......... .......... .......... ..........
Tetraselmis.levis       .......... .......... .......... .......... ..........
Pseudotrebouxia         .......... .......... .......... .......... .........C
Pleurastrum             .......... .......... .......... .......... ..........
Friedmannia             .......... .......... .......... .......... ..........
Microthamnion           .......... .......... .......... .......... ..........
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Myrmecia                GT.GGACCGG AGTAATGATT AAGAGGGACA GTCGGGGGCA TTCGTATTTC
Chlorosarcina           .......... .GTAATGATT AAGAGGGACA GTCGGGGGCA TTCGTATTXX
Pyramimonas.parkae      TCGAGACCGG AGTAATGATT AAGAGGGACA GTTGGGGGCA TTCGTATTTC
Pyramimonas.virgini     TCGGGACCGG AGTAATGATT AAGAGGGACA GTTGGGGGCA TTCGTATTTC
Mesostigma              GTAGGACCGG AGTAATGATT AAGAGGGGTA ATCGGGGGCA TTTGTATTCC
Pedinomonas.tubercu     GTGGGACCGG AGTAATGATT AAGAGGGACA GTCGGGGGCA TTCGTATTTC
Pedinomonas.minor       .......... .......... .......... .......... ..........
Micromonas              .......... .......... .......... .......... ..........
Mantoniella             .......... .......... .......... .......... ..........
Pseudoscourfieldia      .......... .......... .......... .....GGGMM TTSSTATTTC
Nephroselmis.pyrifo     .......... .......... .......... .......... ..........
Pedinomonas.minutis     .......... .......... .......... .......... ..........
Costaria                GCACGTTGTG .GTAATGATT AACAGGAACG GTTGGGGGTA TTCGTATTCA
Saccharomyces           CTAGGACCAT CGTAATGATT AATAGGGACG GTCGGGGGCA TCGGTATTCA
Phaeodactylum           .......... .......... .......... .......... ..........

[                       515                                                564]
Glycine                 .........G GTGAAATTCT TGGATTTATG AAAGACGAAC AACTGCG.AA
Zamia                   ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC CACTGCG.AA
Equisetum               XTTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TRCTGCG.AA
Klebsormidium           .......... .......... .......... .......... ..........
Bryopsis                .......... .......... .......... .......... ..........
Enteromorpha            .......... .......... .......... .......... ..........
Chlamydomonas.eug       CGAGCTAGAG GTGAAATTCT TGGATTTCGG AAAGACCTAC CACTGCG.AA
Chlamydomonas.moe       .......... .TGAAATTCT TGGATTTCGG AAAGACCTAC CACTGCG.AA
Chlamydomonas.rei       .......... .......... .......... .......... ..........
Asteromonas             .......... .......... .......... .......... ..........
Chlorella               AUUGUCAGAG GUGAAAUUCU UGGAUUUAUG AAAGACGAAC UACUGCG.AA
Nanochlorum             AUuGucAGAG GUGAAAUUCU uggauUuAUG AAAgACGAAC UACUGCG.AA
Tetraselmis.carter      .......... .......... .......... .......... ..........
Tetraselmis.levis       .......... .......... .......... .......... ..........
Pseudotrebouxia         ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Pleurastrum             .......... .......... .......... .......... ..........
Friedmannia             .......... .......... .......... .......... ..........
Microthamnion           ....TCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Myrmecia                ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Chlorosarcina           XXXGCTAGAG GTGAAATTCT TGGATTTAXA GAAGACGAAC TTCTGCG.AA
Pyramimonas.parkae      ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Pyramimonas.virgini     ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Mesostigma              GTTGTCAGAG GTGAAATTCT TGGATTTACG GAAGACAAAC ATCTGCG.AA
Pedinomonas.tubercu     ATTGTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Pedinomonas.minor       .......... .......... .......... .......... ..........
Micromonas              .......... .......... .......... .......... ..........
Mantoniella             .......... .......... .......... .......... ..........
Pseudoscourfieldia      ATTXTCAGAG GTGAAATTCT TGGATTTATG AAAGACGAAC TTCTGCG.AA
Nephroselmis.pyrifo     .......... .......... .......... .......... ..........
Pedinomonas.minutis     .......... .......... .......... .......... ..........
Costaria                ATTGTCAGAG GTGAAATTCT TGGATTTATG GAAGACGAAC TACTGCG.AA
Saccharomyces           ATTGTC.GAG GTGAAATTCT TGGATTTATT GAAGACTAAC TACTGCG.AA
Phaeodactylum           .......... .......... .......... .......... ..........

 [                      565                                                614]
Glycine                 AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Zamia                   AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
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Equisetum                AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGXXXG
Klebsormidium            .......... AAGGATGTTT TCATT.AATS ..GAXXX.GA AAGTTGGGGG
Bryopsis                 .......... .......... .......... .......... ..TTGGAGGG
Enteromorpha             .......... .......... .......... .......... .......GGG
Chlamydomonas.eug        AGCATTTGCC AAGGATGTTT TCATT.SATC AAGAAX..GA AAGTAGGGGG
Chlamydomonas.moe        AGCATTTGCC AAGGATGTTT TCATT.SATC AAGAAX..GA AAGTAGGGGG
Chlamydomonas.rei        .......... .......... .......... .........A AAGTTGGGGG
Asteromonas              ..CATTTGCC AAGGATGTTT TCATTCAATC AAXXX...GA AAGTTGGGGG
Chlorella                AGCAUUUGCC AAGGAUGUUU UCAUU.AAUC AAGAAC..GA AAGUUGGGGG
Nanochlorum              AGCAUUUGCC aaGGaUGuUU UCAUU.AAUc aaGAaC..GA AAGUUGGGGg
Tetraselmis.carter       .GCATTTGTC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Tetraselmis.levis        .......... ..GGAYGTTT TCXTT.XATC XAXXXX..GA AAGTTGGGGG
Pseudotrebouxia          AGCATTTGCC AAGGATGTTT TCATT.AATC XXGAAX..GA AAGTTGGGGG
Pleurastrum              .......... .......... .......... .....X..GA AAGTTXGGGG
Friedmannia              .......... .......... .......... .......... ..........
Microthamnion            AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Myrmecia                 AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Chlorosarcina            AGCATTTGCC AAGGATGXTT TCATT.AATC AAGAAC..GA AAGTTAGGGG
Pyramimonas.parkae       AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Pyramimonas.virgini      AGCATTTCC. AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTGGGGG
Mesostigma               AGXATTTGCC AAGGGTACTT TC........ .......... ..........
Pedinomonas.tubercu      AGCATTTGCC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTT.....
Pedinomonas.minor        .......... .......... .......... .......... ......GGGG
Micromonas               .......... .......... .......... .........A AAGTTGGGGG
Mantoniella              .......... .......... .......... .......... ..........
Pseudoscourfieldia       AGCATTTGCC AAGGATGTTT TCATT.GATC AAGAAS..GA AAGTTGGSGG
Nephroselmis.pyrifo      .......... .......... .......... .......... .......GGG
Pedinomonas.minutis      .......... .......... .......... .......... .......GGGG
Costaria                 A.CGTTTACC AAGGATGTTT TCATT.AATC AAGAAC..GA AAGTTAGGGG
Saccharomyces            AGCATTTGCC AAGGACGTTT TCATT.AATC AAGAAC..GA AAGTTAGGGG
Phaeodactylum            .......... .......... .......... .......... ..........

[                        615                                                664]
Glycine                  CTCGAAGACG ATCAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Zamia                    CTCGAAGACG ATCAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Equisetum                CTCGAAGACX ATCAXATACC GT...CCTAG TCTCAACCAT AAACGAKGC.
Klebsormidium            CTCGAAGACG ATCAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Bryopsis                 ATCGAAGATG ATTXXATCCY GT...CGTXG TCCTAMCCGT XAACGATGC.
Enteromorpha             CTCGAAGACG ATTAGATACC GT...CGTAG TCTCAACCAT AAACGAT.C.
Chlamydomonas.eug        CTCGAAGACG ATTAGATACC GT...CGTAG TCTCTACCAT AAACGATGC.
Chlamydomonas.moe        CTCGAAGACG ATTAGATACC GT...CGTAG TCTCTACCAT AAACGATGC.
Chlamydomonas.rei        CTCGAAGACG ATTAGATACC GT...CGTAG TCTCAACCAT AAACGATGC.
Asteromonas              CTCGAAGACG ATTAGATACC GT...CGTAG TCTCAACCAT AAACGATGC.
Chlorella                CUCGAAGACG AUUAGAUACC GU...CCUAG UCUCAACCAU AAACGAUGC.
Nanochlorum              cucgaaGACG AUUAGAUACC GU...Ccuag UCUCAACCAU AAACGAUGC.
Tetraselmis.carter       CTCGAAGACG ATTAGATACC GT...CCTAG TCTCXACCAT XAACGAXXC.
Tetraselmis.levis        CTCGAAGACG ATTAGATACS GT...CCTAG TCTCAACCAT AAACGATSC.
Pseudotrebouxia          CTCGAAGACG ATTAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Pleurastrum              CTCRAAGWCG ATTRGATACC GT...MKTAG WCTCAAYSRT ARWCGATGC.
Friedmannia              .........CG ATTAGATRCC GT...CCTAG TCTCAACCAT AAACGATGC.
Microthamnion            CTCGAAGACG ATTAGATACC GT...CGTAG TCTCAACCAT AAACGATGC.
Myrmecia                 CTCGAAGACG ATTAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Chlorosarcina            CTCGAAGACG ATTAGATACC GT...CGTAG TCTCAACCAT AAACGATGC.
Pyramimonas.parkae       CTCGAAGACG ATCAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
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Pyramimonas.virgini  CTCGAAGACG ATCAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Mesostigma           .......... .......... .......... .......... ..........
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    CTCGAAGACG ATTAGATACC GT...CCTAG TCTCAACCAT AAACGATGC.
Micromonas           CTCGAAGATG ATTAGATASS AT...CCTAG TCTCAACCAT AAACGATGC.
Mantoniella          ......GATG ATTAXATACM AT...CXTAG TCTCXACCAT AAACGGAYXC
Pseudoscourfieldia   CTCGAAGATG ATTAGATACC AT...CGTAG TCTCAACCAT AAACGATGC.
Nephroselmis.pyrifo  CTCGGAGATG ATTAGKKXCC AT...CGTAG TCTCAACCAT AAACGATGC.
Pedinomonas.minutis  ATCGAAGACG ATCAGATACC XT...CXTAG TCTCTACCAT AAACTATXC.
Costaria             ATCGAAGATG ATTAGATACC AT...CGTAG TCTTAACCAT AAACTATGC.
Saccharomyces        ATCGAAGATG ATCTGGTACC GT...CGTAG TCTTAACCAT AAACTATGC.
Phaeodactylum        .TCGAAGATG ATTAGATACC AT...CGTAG TCTTAACCAT AAACTATGC.

[                    665                                                714]
Glycine              .CGACC.AGG GATCAGC..G GATGTTGCTT TT.AG..GAC TCCGCTGGCA
Zamia                .CGACC.AGG GATCGGC..G GATGTTGCTC TA.AG..GAC TCCGCCGGCA
Equisetum            .XGACT.AGG GATTCGX..X GATGTTACTT CA.AT..GAC TCTGCCGGCA
Klebsormidium        .CGACT.AGG GATTGGC..G GATGTTAATT TG.AT..GAC TCCGCCAGCA
Bryopsis             .CSTCT.SGG GATCTGT..C AKAGTTTAAG GXXATCTGAC TCGACGGCAC
Enteromorpha         .CGACC.AGG GATTGCC..G GGTGTTTTTT TG.AT..GAC TCCGCCAGCA
Chlamydomonas.eug    .CGACC.AGG GATTGGC..A GGTGTTCCTT TG.AT..GAC CCTGCCAGCA
Chlamydomonas.moe    .CGACC.AGG GATTGGC..A GGTGTTCCTT TG.AT..GAC CCTGCCAGCA
Chlamydomonas.rei    .CGACT.AGG GATTGGC..A GATGTTCTTT TG.AT..GAC TCTGCCAGCA
Asteromonas          .CGACT.AGG GATTGGC..A GGTGTTTCGG TTGAT..GAC CCTGCCAGCA
Chlorella            .CGACU.AGG GAUCGGC..G GAUGUUUCUU CG.AU..GAC UCCGCCGGCA
Nanochlorum          .CGACUXAGG GaUCGGC..G GGuGUUUUUU UG.AU..GAC CCCGCCGGca
Tetraselmis.carter   .CGACX.AGG GATTGGC..A GASSTTXXXT TX.AT..GXX TCTGCCAGCA
Tetraselmis.levis    .CGACT.AGG GATTGGC..A GACXXTTTTT TG.AT..GAC TCTGCCAGCA
Pseudotrebouxia      .CGACT.AGG GATTGGC..G GGTGTTCTTT CG.AT..GAC CCCGCCAGCA
Pleurastrum          .CKAMT.AGG KATTGTGCCK GATGTTTATT CA.AT..TAC TCCGCCAKCA
Friedmannia          .CKACT.AGG GATTXCK..R XXTGTTCTXX XX.XK..GAC CCCGCCAGCA
Microthamnion        .CGACT.AGG GATTGGC..G GATGTTTTTT CG.AT..GAC TCCGCCAGCA
Myrmecia             .CGACT.AGG GATT...... .......... .......... ..........
Chlorosarcina        .CGACT.AGG GATCGGC..G GATGTTCCTT TG.AT..GAC TCCGCCGGCA
Pyramimonas.parkae   .CGACT.AGG G......... .......... .......... ..........
Pyramimonas.virgini  .CGACT.AGG GATTGGC..G GXTGTTATAT CG.AT..GXC TTCGCCAG..
Mesostigma           .......... .......... .......... .......... ..........
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    .CGACT.AGG GATTGGC..G GATGTTGATT CG.AT..GAC TTCGCCAGCA
Micromonas           TCGACT.AGG GAXXGGC..G GATGTTAATT .G.AT..GAC TCCGCMAGCA
Mantoniella          YGXACW.AKR GATTCAM..C XATCTTAATX .X.AT..X.C TCXXCCAXCA
Pseudoscourfieldia   .CGACT.AGG GATTGGS..A GATGTTAGTT CG.AT..GAC TCTGCCAGCA
Nephroselmis.pyrifo  .CGACT.AGG GATTGGC..A GATGTTAGTT CG.AT..GAC TCTGCCAGCA
Pedinomonas.minutis  .CGACT.AGG GATTGGT..G GACGTTGTTT TWTCC..GAC TCCATCAGCA
Costaria             .CGACT.AGG GATTGGC.GG TTCGTTAATT TACAG..GAC TCCGTCAGCA
Saccharomyces        .CGACT...A GATCGGG..T GGTGTTTTTT TA.AT..GAC CCACTCGGTA
Phaeodactylum        .CGACA.AGG GATTGGC... GGGGTTTCGT TAC....GTC TCCGTCAGCA

[                    715                                                764]
Glycine              CCTTATGAGA AATCA.AAGT CTTTGGG.TT CC..GGGGGG AGTATGGTCG
Zamia                CCTTATGAGA AATCA.AAGT TTTTGGG.TT CC..GGGGGG AGTATGGTCG
Equisetum            CCTTATGAGA AATCA.AAGT CTTTGGG.TT CC..... .....
Klebsormidium        CCTTATGAGA AATCX.XAGT TTTTGGG.TT CC..GGGGGG AXTATGGXTC
Bryopsis             CCTT.CGAGA AATCA.AAGA CTATGGGCTT CC..GGGGAT AXXATGGTG.
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Enteromorpha          CCTCATGAGA AATCA.AAGT CTTTGGG.TT CC..GGGGGG AGXATGG...
Chlamydomonas.eug     CCTTGAGAGA AATCA.GAGT CTTTGGG.TT CC..GGGGGG AGTATGG...
Chlamydomonas.moe     CCTTGAGAGA AATCA.GAGT CTTTGGG.TT CC..GGGGGG AGTATGG...
Chlamydomonas.rei     CCTTATGAGA AATCA.AART TTTTGGG.TT CC..GGGGGG AGTATGGTCX
Asteromonas           CCTTATGAGA AATCA.AAGT TTTTGGG.TT CC..GGGGGG AGTAT.....
Chlorella             CCUUAUGAGA AAUCA.AAGU UUUUGGG.UU CC..GGGGGG AGUAUGGUCG
Nanochlorum           CCUUAUGAGA AAUCA.AAGU UuuugGG.UU CC..GgGGGG AGUaUGGUCG
Tetraselmis.carter    CCTTATGAGA AATXA.AAGT TTTTGGG.TT .......... ..........
Tetraselmis.levis     CCTTATGAGA AATCX.XAGT TTTTGGG.TT CC..GGGGGG AGTA......
Pseudotrebouxia       CCTTATGAGA AATCX.XAGT TTTTGGG.TT CC..GGGGGG AGTATGG...
Pleurastrum           CCTYATGAXA AATCX.AAGW WTTTRSK.TT CC..SKGGGG AGTATGS...
Friedmannia           CCTTATGAGA AATCA.AAGT TTTTGGS.TT CC..GTXGXG AGTATGGTCC
Microthamnion         CCTTATGAGA AATCX.XAGT TTTTGGG.TT CC..GGGGGG AGTATGGTC.
Myrmecia              .......... .......... .......... .......... ..........
Chlorosarcina         CCTTATGAGA AA........ .......... .......... ..........
Pyramimonas.parkae    .......... .......... .......... .......... ..........
Pyramimonas.virgini   .......... .......... .......... .......... ..........
Mesostigma            .......... .......... .......... .......... ..........
Pedinomonas.tubercu   .......... .......... .......... .......... ..........
Pedinomonas.minor     CCTTATGAGA AATCA.AAGT TTTTGGG.TT CCCGTGGGGG AGTAT.....
Micromonas            CCTTATGAGA AATCA.AAXT TTTTGGG.TT CC..XXXGGG A.........
Mantoniella           CCTTATXAMA AATCM.AAXT TTTT...... .......... ..........
Pseudoscourfieldia    CCTTATGAGA AATCA.AAGT TTTTGGG.TT CC..XGGGGG AXTAT.....
Nephroselmis.pyrifo   CCTTATXAGA AATCX.AAGT TTTTGGG.TT CC........ ..........
Pedinomonas.minutis   CCTTATGAGA AATCA.AAGT CTTT...... .......... ..........
Costaria              CCTTCCGAGA AATCA.AAGT CTTTGGG.TT CC..GGGGGG AGTATGGTCG
Saccharomyces         CCTTACGAGA AATCA.AAGT CTTTGGG.TT CT..GGGGGG AGTATGGTCG
Phaeodactylum         CCTTATGAGA AATCACAAGT CTTTGGG.TT CC..GGGGGG AGTATGG...

18J Primer
[                     765                                                814]
Glycine               .......... .......... .......... .......... ..........
Zamia                 TGGAGCCTGC GGCTTAATTT GACTCAACAC GGGGAAACTT ACCAGGTCCA
Equisetum             .......... .......... .......... ....AAACTT ACCAGGTCCA
Klebsormidium         .......... .......... .......... .......... ..........
Bryopsis              .......... .......... .......... .......... ..........
Enteromorpha          .......... .......... .......... ....AAACTT ACCAGGTCCA
Chlamydomonas.eug     .......... .......... .......... .......... ..........
Chlamydomonas.moe     .......... .......... .......... .......... ..........
Chlamydomonas.rei     .......... .......... .......... .......... ..........
Asteromonas           .......... .......... .......... .......... ..........
Chlorella             UGGAGCCUGC GGCUUAAUUU GACUCAACAC GGGAAAACUU ACCAGGUCCA
Nanochlorum           UGGAGCcUGC GGCUUAAUUU GACUCAACAC GGGAAAACUU ACCAGGuCCA
Tetraselmis.carter    .......... .......... .......... .......... ..........
Tetraselmis.levis     .......... .......... .......... .......... ..........
Pseudotrebouxia       .......... .......... .......... .......... ..........
Pleurastrum           .......... .......... .......... .......... ..........
Friedmannia           .......... .......... .......... .......... ..........
Microthamnion         .......... .......... .......... .........T ACCAGGTCCA
Myrmecia              .......... .......... .......... .......... ..........
Chlorosarcina         .......... .......... .......... .......... ..........
Pyramimonas.parkae    .......... .......... .......... .......... ..........
Pyramimonas.virgini   TGGCAG.TGC GGCTTAATTT GACTCAACAC GGG.AAACTT ACCAGGTCCA
Mesostigma            TGCGAG.TGC GGCTTAATTT GACTCAACAC GGGGAAACTC ACCAGGTCCA
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Pedinomonas.tubercu ..... ..... ..... ..... .....
Pedinomonas.minor   ..... ..... ..... ..... .....
Micromonas          ..... ..... ..... ..... .....
Mantoniella         ..... ..... ..... ..... .....
Pseudoscourfieldia  ..... ..... ..... ..... .....
Nephroselmis.pyrifo ..... ..... ..... ..... .....
Pedinomonas.minutis ..... ..... ..... ..... .....
Costaria            TGGAGCCTGC GGCTTAATTT GACTCAACAC GGGGAAACTT ACCAGGTCCG
Saccharomyces       TGGAGCCTGC GGC.TAATTT GACTCAACAC GGGGAAACTC ACCAGGTCCA
Phaeodactylum       ..... ..... ..... ..... .....

[                   815                                              864]
Glycine             ........AA GGATTGACAG ACTGAGAGCT CTTT.CTTGA TTCTATGGGT
Zamia               GACATAGCAA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTCTATGGGT
Equisetum           GACATAGTAA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTCTATGGGT
Klebsormidium       ..... ..... ..... ..... .....
Bryopsis            ..... ..... ........CT CTTT.CTTGA TTC.CTKGAT
Enteromorpha        GACATGGAR. .GATTGACAG ATTGAGAGCT CTTT.CTTGG TTCTATGGGT
Chlamydomonas.eug   ..... ..... ..... .TTT..TTGA TTCTXTGGGT
Chlamydomonas.moe   ..... .....AG ATT.AGAGCT CTTT..TTGA TTCTXTGGGT
Chlamydomonas.rei   ..... ......ASAG ATTGAGAGCT CTTT.CTTGA TTCTXTXGGT
Asteromonas         ..... .....GACAG ATTGAGAGCT CTTT.CTTGA TTCTGTGGGT
Chlorella           GACAUAGUGA GGAUUGACAG AUUGAGAGCU CUUU.CUUGA UUCUAUGGGU
Nanochlorum         GACAUAGuGA ggauuGAcAG AUUGAGAGCU CUuU.Cuuga UUCUAUGGGU
Tetraselmis.carter  ..... ..... ..... ..... ...TATGGGT
Tetraselmis.levis   ..... ..... ..... ..... ...TATGGGT
Pseudotrebouxia     ..... ..... ..... ..... .....
Pleurastrum         ..... ..... ..... ..... .....
Friedmannia         ..... ..... ..... ..... .....
Microthamnion       GACATAGT.A GGAXXGACAG ATTGAGAGCT CTTT.CTTGA TTCTMTSGGT
Myrmecia            ..... ..... ..... ..... .....
Chlorosarcina       ..... ..... ..... ..... .....
Pyramimonas.parkae  ..... ..... ..... ..... .....
Pyramimonas.virgini GACATAGTAA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTCTATGGGT
Mesostigma          GACATAGTAA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTCTATGGGT
Pedinomonas.tubercu ..... ..... ..... ..... .....
Pedinomonas.minor   ..... ..... ..... ..... ......GGGT
Micromonas          ..... ..... ..... ..... .....
Mantoniella         ..... ..... ..... ..... .....
Pseudoscourfieldia  ..... ..... ..... ..... .....
Nephroselmis.pyrifo ..... ..... ..... ..... .....
Pedinomonas.minutis ..... ..... ..... ..... .....
Costaria            GACATAGTGA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTCTATGGGT
Saccharomyces       GACACAATAA GGATTGACAG ATTGAGAGCT CTTT.CTTGA TTTTGTGGGT
Phaeodactylum       ..... ..... ..... ..... ..C.RTGGGT

[                   865                                              914]
Glycine             GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGAGC..GA TTTGTC..TG
Zamia               GGTGGT.GCA TGGCGGTT.C TTA.GTTGGT .GGAGC..GA TTTGTC..TG
Equisetum           GGTAGT.GCA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TTTGTC..TG
Klebsormidium       ..... ..... ..... ..... .....
Bryopsis            GTTXGT.GCA TGGCCGAT.C TCA...CCCT .GGGTT..GA CTTXTC..AG
Enteromorpha        GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Chlamydomonas.eug   GGTXGT.GCX TGGCCGTT.C TTX.GTTGGT .GGGTT..GC CTTGTC..AG
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Chlamydomonas.moe    GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Chlamydomonas.rei    GXTXXT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Asteromonas          GGTXGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Chlorella            GGUGGU.GCA UGGCCGUU.C UUA.GUUGGU .GGGUU..GC CUUGUC..AG
Nanochlorum          GGuGGU.gcA UGGCCGUU.c UUA.GUUgGU XGGGUU..GC CUUGUC..AG
Tetraselmis.carter   GXTXGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Tetraselmis.levis    GXTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Pseudotrebouxia      .......... ..GCCGTT.C TTA.GTTGGT .GGGXT..GC CTTGTC..AG
Pleurastrum          ..TGGT.GCA TGGCCGTT.C TTA.GTTGGT GGGGTT..GC CTTGTC..AG
Friedmannia          ...GGT.XGA TGGC.GTT.C TTA.GTTGGT .GGGTT..GC CTTXXC..AG
Microthamnion        GGTXGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Myrmecia             .......... .......... .......... .......... ..........
Chlorosarcina        .......... .......... .......... .......... ..........
Pyramimonas.parkae   .......... .......... .......... .......... ..........
Pyramimonas.virgini  GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TTTGTC..TG
Mesostigma           GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TT.GTC..TG
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    GGTKGT.GCA TGGCCGTT.C TTA.GTTGGT .GGGTT..GC CTTGTC..AG
Micromonas           ..TXGT.GCA TGGCCGTT.C TTX.GTTGGT .GGAGT..GA TTTGTC..TG
Mantoniella          ..TXGX.GCW TGGCCGTT.S TTX.GTTGGT .GGAGT..GA TTSSTC..TG
Pseudoscourfieldia   ..TGGC.GCA TGGCCGTT.C TTA.GXTGGT .GGAGT..GA TTTGTC..TG
Nephroselmis.pyrifo  ........CA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TTTGTC..TG
Pedinomonas.minutis  .......GCA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TTTGTC..TG
Costaria             GGTGGT.GCA TGGCCGTT.C TTA.GTTGGT .GGAGT..GA TTTGTC..TG
Saccharomyces        GGTGGT.GCA TGGCCGTTTC TCA.GTTGGT .GGAGT..GA TTTGTC..TG
Phaeodactylum        GGTXGT.GSA TGGCCGTTGC TWA.GTTGGT .GGAGT..GA TTTGTC..TG

[                    915                                                964]
Glycine              GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAA TAGCT.ATT.
Zamia                GTTAATTCCG TTAACGAACG AGACCTC.GG .CCTGCTAAC TAGCT.ACGC
Equisetum            GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAC TAGTT.ACGC
Klebsormidium        .......... ..AACGAACG AGACCTC.AG .CCTGCTAAC TAGTT.ACAC
Bryopsis             GTTCACTCCG KTAAYXYXYG AGACCCC.GA .CCT.CCAAA TAGCA.CCTC
Enteromorpha         GTTGATTCCG GTAACGAASG AGACCTC.AG .CCTGCTAAA TAGTG.ACGA
Chlamydomonas.eug    GTTGATTCCG GTXACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.GGCG
Chlamydomonas.moe    GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.GGCG
Chlamydomonas.rei    GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.AGCA
Asteromonas          GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTACTAAA TAGTC.GCGC
Chlorella            GUUGAUUCCG GUAACGAACG AGACCUC.AG .CCUGCUAAA UAGUC.ACGG
Nanochlorum          GUUGAUUCCG GUAACgAACG AGACCUC.AG XCCUgCUAAc uAGUC.ACGC
Tetraselmis.carter   GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTT.ACTC
Tetraselmis.levis    GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTT.ACTC
Pseudotrebouxia      GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ACGG
Pleurastrum          GTTGATTCCG GTAACGAAGG AGACCTC.AG .CCTGCTAAA TAGTC..CTA
Friedmannia          GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ACGG
Microthamnion        GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ACTA
Myrmecia             .......... .......... .......... .......... ..........
Chlorosarcina        .......... .......... .......... .......... ..........
Pyramimonas.parkae   .......... .......... .......... .......... ..........
Pyramimonas.virgini  GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAA TAG.......
Mesostigma           GTTAATTCCG .......... .......... .......... ..........
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    GTTGATTCCG GTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTCCC..S
Micromonas           GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ATAC
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Mantoniella           GTTAATTCCG TTAACKAACK AGACCTC.WG .CCTGCTAAA TAGTC.ATAC
Pseudoscourfieldia    GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ACGC
Nephroselmis.pyrifo   GTTAATTCCG TTAACGAACG AGACCTC.AG .CCTGCTAAA TAGTC.ACGC
Pedinomonas.minutis   GTTAATTCCG TTAACGAACG AGACCTC.GA .CCTGCTAAA TAGGTTC.AG
Costaria              GTTAATTCGT TAACGAACGA GACCCCC..G .CCTGCTAAA TAGTGT.GGC
Saccharomyces         CTTAATTGCG ATAACGAACG AGACCTT.AA .CCTACTAAA TAGTGG.TGC
Phaeodactylum         GTTAATTCCG TTAACGRACG AGACCCC.TG .CCTGCTAAA TAGCCC.AGT

[                     965                                                1014]
Glycine               .GAGG..TAA C.CCTCCACG .GCC.AGCTT C.TTAGAGGG A..CTAT..G
Zamia                 GGAGG..GTT T.CTTTCGTG .GCC.AGCTT C.TTAGAGGG A..CTAT..G
Equisetum             GAAGA..CTT G.TCTTCGTG .GCC.AACTT C.TTAGAGGG A..CTAT..G
Klebsormidium         GGAGA..TTC T.TCTCCGTG .GCC.AACTT C.TTAGAGGG A..CTATT.T
Bryopsis              GG.....CAT X.CCGGG.TG CXGC..GCTT X.TTGGAGGG A..CTTCC.G
Enteromorpha          TTGC.TCG.. GCAGTCGCG. .....CGCTT S.TTAGAGGG A..CTGTT.G
Chlamydomonas.eug     GTCC.TTTCT GGATCGCCX. ....CGACTT C.TTAGAGGG A..CTATT.G
Chlamydomonas.moe     GTCC.TTTCT GGATCGCCT. ....CGACTT C.TTAGAGGG A..CTATT.G
Chlamydomonas.rei     .XXXXXXXXG CGGTGCGCC. .....GACTT C.TTAGAGGG A..CTATT.G
Asteromonas           XTAC.GCTTG TAGACGCCT. .....GGCTT C.TTAGAGGG A..CTATT.G
Chlorella             UUGG.UUCGC CAGCCGGCG. .....GACUU C.UUAGAGGG A..CUAUU.G
Nanochlorum           GUGC.UCCGG CACGCGGCG. .....GACUu c.UUAGAGGG A..CUAUU.G
Tetraselmis.carter    CTAC.TTTGG TAGGAGGTG. .....AACTT X.TTAGAGGG A..CTATT.G
Tetraselmis.levis     CTAC.TTTGG TAGGAGGTG. .....AACTT X.TTAGAGGG A..CTATT.G
Pseudotrebouxia       CTGC.TTT.Y CAGTCGGCA. .....GACTT X.TTAGAGGG A..CTTTT.G
Pleurastrum           ATXCTTCTCG CGGTTAGCT. .....GACTT GGTTAGAGGG A..CTATT.G
Friedmannia           TTGC..XXXX CAGCCGGCCG .....AGCTT C.TTAGAGGG A..CTTTT.G
Microthamnion         TCACTTCTTG TGGTAGGXA. .....GACTT C.TTAGAGGG A..CTATT.G
Myrmecia              .......... .......... .......... .......... ..........
Chlorosarcina         .......... .......... .......... .......... ..........
Pyramimonas.parkae    .......... .......... .......... .......... ..........
Pyramimonas.virgini   .......... .......... .......... .......... ..........
Mesostigma            .......... .......... .......... .......... ..........
Pedinomonas.tubercu   .......... .......... .......... .......... ..........
Pedinomonas.minor     TGACATATGT C...AGCAGG C....GACTT C.TTAGAGGG A..CTATT.G
Micromonas            GCT...ACTC T..TAGCGCA ...GTGACTT C.TTAGAGGG A..CTATG.T
Mantoniella           GCT...ACTC T..TAGCGCA ...GTGACTT C.TTAGAGGG A..CTATG.T
Pseudoscourfieldia    GATGC..TCC T.GCATGGCG GCG..GACTT C.TTAGAGGG A..CTATC.G
Nephroselmis.pyrifo   GATGC..TCC T.GCATGGCG GCG..GACTT X.TTAGAGGG A..CTATC.G
Pedinomonas.minutis   TATCGA.TGC G.TCGATGCT TG..GAGCTT X.TTAGAGGG A..CTXTC.G
Costaria              TTACGCTTCT GTGTAGGTGC T....CGCTT C.TTAGAGGG A..CTTTC.G
Saccharomyces         TAGC...ATT T.GCTGGTTA T...CCACTT C.TTAGAGGG A..CTATC.G
Phaeodactylum         GRGTGA.ATX .TCACTGACC A...GGGCTT C.TTAGAGGG A..CGTGC.G

[                     1015                    1044]
Glycine               GCCG.C.TTA GG.CC.AC.G GAAGTTTGAG
Zamia                 GCCG.T.TTA GG.CC.AT.G GAAGTTTG..
Equisetum             GCCG.T.CTA GG.CC.AT.G G.........
Klebsormidium         GGCG.T.CTA CAGCCAAT.G GAAXTTTGA.
Bryopsis              GTG..A.GAA ...CC....G GTTXAA....
Enteromorpha          GCG..T.CTA G..CCAAT.G GAAGTAT...
Chlamydomonas.eug     ACG..T.TTA G..TCAAT.G GAA.......
Chlamydomonas.moe     ACG..T.TTA G..TCAAT.G GAAG......
Chlamydomonas.rei     GCG..T.TTA G..CCAAT.G GAAXTAT...
Asteromonas           GCG..T.TTA G..CCAAT.G GAA.......
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Chlorella              GCG..A.CUA G..CCAAU.G GAAGCAUGAG
Nanochlorum            GCG..A.CUA G..CCAAU.G GAAGCaUGAG
Tetraselmis.carter     GCG..T.TTX G..CCAAT.G GAAGTXTA..
Tetraselmis.levis      GCG..T.TTX G..CCAAT.G GAAGTGT...
Pseudotrebouxia        GCG..A.CTA G..CCAAA.G GAAGTGTG..
Pleurastrum            GCG..T.TTX G..TCAAT.G GAAXTAT...
Friedmannia            GCG..CCXXX G..CCAAA.G GAA.......
Microthamnion          TCG..T.TTA G..GCAAT.G GA........
Myrmecia               .......... .......... ..........
Chlorosarcina          .......... .......... ..........
Pyramimonas.parkae     .......... .......... ..........
Pyramimonas.virgini    .......... .......... ..........
Mesostigma             .......... .......... ..........
Pedinomonas.tubercu    .......... .......... ..........
Pedinomonas.minor      CCG..T.GTA G..GCAAT.G GAA.......
Micromonas             GCG..T.TTX G..CACAT.G GAAXTT....
Mantoniella            GCG..T.TTW G..CACAT.G GAAXTTTGA.
Pseudoscourfieldia     STS..A.TTX A..CCGAT.G GAA.......
Nephroselmis.pyrifo    GTS..A.TTX A..CCGAT.G GA........
Pedinomonas.minutis    GAT..C.CXX A..CCGGT.G GAAGT.....
Costaria               GTG..A.CTA A..CCGAA.. GAAGTTGGGG
Saccharomyces          GTT..T.CAA G..CCGAT.G GAAGTTTGAG
Phaeodactylum          TTC..TATTA G..ACGCA.G GAAGATAGGG

18L Primer
[                      1045                                                1094]
Glycine                ATTGTTGGTC TTCAACG.AG GAATTCCTA. GTAA..GCGA G..TCATCA.
Zamia                  ATTATTGATC TTCAACG.AG GAATTCCTA. GTAAGCGCGA G..TCATCA.
Equisetum              ATTATTGATC TTCAACT.AG GAATTCCTA. GTAAGCKCGA G..TCATCA.
Klebsormidium          .......... .......... .......... .......... ..........
Bryopsis               .......... .......... GAATGCCTA. GTAGTCGTGG G..TCGGTA.
Enteromorpha           .......... .......... .....CCTA. GTAAGCGCGA G..TCATCA.
Chlamydomonas.eug      .......... .........G GAATGCCTA. GTXAGCGTGA G..TCATCA.
Chlamydomonas.moe      .......... .........G GAATGCCTA. GTAAGCGTGA G..TCATCA.
Chlamydomonas.rei      .......... .......... .........GA G..TCATCA.
Asteromonas            .......... .......... GAATGCCTA. GTAAGCGTGA G..TCATCA.
Chlorella              AUUAUUAAUC UUCAACG.AG GAAUGCCUA. GUAAGCGCAA G..UCAUCA.
Nanochlorum            AUUAUUAAUC UUCAACG.AG GAAUGCCUA. GUAAGCGCAA G..UCAUCA.
Tetraselmis.carter     .......... .......... .......... .......... ...TCATCA.
Tetraselmis.levis      .......... .......... ...TGCCTA. GTAAGCGTGA ...TCATCA.
Pseudotrebouxia        .......... .......... .....CCTA. GTAA..GCGA G..TCATCA.
Pleurastrum            .......GTC TTCAACG.AG GAATGCCTA. GTAA..GCGA G..TCATCA.
Friedmannia            .......... .........CG GAATGCCTA. GTAAGCGCGA G..TCXXXA.
Microthamnion          .......GTC TTCAACG.XG GAATGCCTA. GTAAGCGCGA G..TCATCA.
Myrmecia               .......... .......... .......... .......... ..........
Chlorosarcina          .......... .......... .......... .......... ..........
Pyramimonas.parkae     .......... .......... .......... .......... ..........
Pyramimonas.virgini    .......... .......... .......... .......... ..........
Mesostigma             .......... .......... .......... .......... ..........
Pedinomonas.tubercu    ATTATTGATC TTCAACG.AG GAATGCCTA. GTAAGCGCGA G..TCATCA.
Pedinomonas.minor      .......... .......... .......... .......... ..........
Micromonas             .......... .........AG XAATGCCTX. GTAAGCGCAA G..TCATCA.
Mantoniella            .......... .......... .......... .......... ..........
Pseudoscourfieldia     .......... .......... .......... .......... ..........
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Nephroselmis.pyrifo ..... ........AG GAATGCCTX. GTAA..GCGA G..TCATCA.
Pedinomonas.minutis ATTATTGATC TTGAACG.AG GAATTC.... ..... .....
Costaria            ATTATTGCTC TTCAACG.AG GAATTCCTA. GTAAGCGCAA G..TCATCA.
Saccharomyces       ..... ..... ..... .XAAACGCAG A..TCATCAA
Phaeodactylum
[                   1095                                                1144]
Glycine             GCTCGCGTTG ACT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Zamia               GCTCGCGTTG ACT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Equisetum           GCTCGCGTTG ACT.AY.GTC CCTGCCCTTT GTACACACTG CCC.GTCGCT
Klebsormidium       ..TCGCGTTG ATTTA..GTC CCTGCCCTTT GTACACACXG CCC.GTCGCT
Bryopsis            TCCCACGACG ATT.XC.GTC CCTGCCCTTT GTACGCRCSG CCC.GTCGCT
Enteromorpha        TCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Chlamydomonas.eug   GCTCGCGTTG ATT.XC.GTC CCTGCCCTTT GTXCWCACCG CCC.GTYGCT
Chlamydomonas.moe   GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACWYWXCG CCC.GTYGCT
Chlamydomonas.rei   GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Asteromonas         GCTCACGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Chlorella           GCUUGCGUUG AUU.AC.GUC CCUGCCCUUU GUACACACCG CCC.GUCGCU
Nanochlorum         GCUUGCGUUG AUU.AC.GUC CCUGCCCUUU GUaCACACCG CCC.GUCGCU
Tetraselmis.carter  GATCGCGTTG ATT.AX.GTC CCTGCCCTTT GTACACA.CX CCC.GTCGCT
Tetraselmis.levis   GATCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACA.CG CCX.GTCGCT
Pseudotrebouxia     GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACA.CG CCC.GTCGCT
Pleurastrum         GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Friedmannia         GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Microthamnion       GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Myrmecia            ..... ..... ..... ..... .....
Chlorosarcina       ..... ..... ..... ..... .....
Pyramimonas.parkae  ..... ..... ..... ..... .....
Pyramimonas.virgini ..... ..... ..... ..... .....
Mesostigma          ..... ..... ..... ..... .....
Pedinomonas.tubercu GCTCGCGTTG ATT.AC.GTC CCTGCCCTTT GTACAGACCG CCC.GTCGCT
Pedinomonas.minor   ..... .....C CCTGCCCTTT GTACACA.CG CCC.GTCGCT
Micromonas          GCTTGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCX CCC.GTCGCT
Mantoniella         ..... ..... ..... ..... .....
Pseudoscourfieldia  ..... ..... ..... ..... .....
Nephroselmis.pyrifo GCTCGCGTTG ATT.AX.GTC CCTGCCCTTT GTACACA.CX CCC.GTCGCT
Pedinomonas.minutis ..... .....GTC CCTGTCCTTT GTACRCA.CG CCC.GT.GCT
Costaria            ..... ..... ..... ..... .....
Saccharomyces       GCTTGCGTTG ATT.AC.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCT
Phaeodactylum       TCTGCCATTG ATT.AX.GTC CCTGCCCTTT GTACACACCG CCC.GTCGCA

[                   1145                                                1194]
Glycine             CCTACCGATT .GAAT.GGT. CC.GGTGAAG TGTTCGGAT. .TGCGGCGAC
Zamia               CCTACCGATT .GAAT.GAT. CC.GGTGAAG TGTTCGGAT. .CGTCGCGAC
Equisetum           CCTACCGATT .GAAT.GGT. CC.GGTGAAG TTTTCGGAT. .T.CGGCGAC
Klebsormidium       CCTACCGATT .GAAT.GAT. CC.GGTGAAG TTTTCGGAT. .TGCGGCTAC
Bryopsis            GATCCTGAT. .GGCA.GCR. TT.GRCGRRR TCGGGGGAG. .CGG..AGTG
Enteromorpha        CCTACCGATT .GAAC.GTG. CT.GKKGAAG CGTTAGGAC. .TGG..AACC
Chlamydomonas.eug   CCTACCGATT .GGGT.GTG. CT.GGTGAAG TGTTCGGAT. .TGG..CTTX
Chlamydomonas.moe   CCTACCGATT .GGGT.GTG. CT.GGTGAAG TGTTCGGAT. .TGG..CTTT
Chlamydomonas.rei   CCTACCGATT .GGGT.GTG. CT.GGTGAAG TGTTCGGAT. .TGA..GCTT
Asteromonas         CCTACCGATT .GGGT.GTG. CT.GGTGAAG TGTTTGGAT. .TGG..CGCC
Chlorella           CCUACCGAUU .GGGU.GUG. CU.GGUGAAG UGUUCGGAU. .UGG..CGAC
Nanochlorum         CCUaCCgaUu .GGGU.GUG. cu.GGUgaAA UGcuCggaU. .UGG..CGGC
Tetraselmis.carter  CCTACCGATT .GAAT.GTG. TT.GGTGAGG AGTTXGGAT. .TGG..CAGT
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Tetraselmis.levis     CCTACCGATT .GAAT.GTG. TT.GGTGAGG AGTTCGGAT. .TGG..CAGT
Pseudotrebouxia       CCTACCGATT .GGGT.GTG. CT..GTGAAG CGTTCGGAT. .TGC..GTTA
Pleurastrum           CCTACCGATT .GGGT.GTG. CT.GGTGAAA AGTTTGGAC. .TGG.CGGTA
Friedmannia           CCTACCGATT .GGAT.GTG. CT.GGTGAAG TGTTCGGAT. .TG...CGGC
Microthamnion         CCTACCGATT .GGGT.GTG. CT.GGTGAAG CGTTCGGAC. .TGA.GCGCG
Myrmecia              .......... .......... .......... .......... ..........
Chlorosarcina         .......... .......... .......... .......... ..........
Pyramimonas.parkae    .......... .......... .......... .......... ..........
Pyramimonas.virgini   .......... .......... .......... .......... ..........
Mesostigma            .......... .......... .......... .......... ..........
Pedinomonas.tubercu   CCTACCGATT .GAAT.GTG. CT.GGTGAAG XGTTGGTRT. .CGATGCATG
Pedinomonas.minor     CCTACCGATT .GGGT.GTG. CT.GGTGAAG CGTTAGGAT. .TGACGCAGG
Micromonas            CCTACCGATT .GAAT.GGT. CC.GGTGAAG CGTTCGGAC. .CGT..GGCT
Mantoniella           .......... .......... .......... .......... ..........
Pseudoscourfieldia    .......... .......... .......... .......... ..........
Nephroselmis.pyrifo   CCTACCGATT .GAAT.GTG. CT.GGTKAGG AGTCCGGAT. .KAT...GCG
Pedinomonas.minutis   ACTACCGATT .GAAT.CXT. TT.GGTGAGG CTCACGGAC. .TGTCGTGCT
Costaria              .......... .......... .......... .......... ..........
Saccharomyces         AGTACCGATT .GAAT.GGC. TT.AGTGAGG CCTCAGGAT. .CTGCTTAGA
Phaeodactylum         CCTACCGAKT .GGAT.GGT. CC.GGTGAAG CCTCGGGAT. .TGT..GACC

[                     1195                                                1244]
Glycine               GTGAGCGG.. TTCG.CTGCC CGCGACGTTG T..GAGAA.G TCCACT..GA
Zamia                 GACGGCGG.. TTCG.CTGGG CGCGACGTCG C..GAGAA.G TTCATT..GA
Equisetum             GCTGGCGG.. TXCG.CCGGC ...GACGTTG T..GAGAA.G TTCATT..GA
Klebsormidium         ..TGGTCC.. GCCG.CCGAA GAAGCTGTGA G..GCAAG.G TTCATT..AA
Bryopsis              GCCGGC.... TTCG.GGTGC GAGCGAGCCG ...GAGAG.C CTGCT...AA
Enteromorpha          TTGGGCCGG. TCTC.CTGCC CATGGTTTC. ...XGSAA.T TTCGTT..XA
Chlamydomonas.eug     GAGGGGTGG. CAASXCTCCC CAG.AGCC.. ...GAGAA.S ATCATT..AA
Chlamydomonas.moe     GAGGGGTGG. CAAXXCTCCC CAG.AGCC.. ...GAGAA.G ATCATT..AA
Chlamydomonas.rei     GGCTGGGG.. CAAC.CTGGC CTTGCTT... ...GAGAA.G TTCATT..AA
Asteromonas           TGCTKGGGG. AAAC.CTCCT CTGGTGCT.. ...GAGAA.G AACATT..AA
Chlorella             CUGGGGCGG. UCUC..CGCU CUCGGCCGCC ...GAGAA.G UUCAUU..AA
Nanochlorum           UuuGGGCGG. UUUC..CGCC CuuGGCUGUC ...GAgaa.G uuCAUU..aa
Tetraselmis.carter    TTGTGGTGG. TTCG.CCAXC TGCTTACAGC T..GAGAA.G TTCTCC..AA
Tetraselmis.levis     TTGTGGTGG. TTCG.CCA.C TGCTTACAGC T..GAGAA.G TTCTCC..AA
Pseudotrebouxia       GTCGGGT... TTTCC...GC CTCCTCTCAC T..GAGAA.G TTCGTT..AA
Pleurastrum           GGCGGGTGG. TTCG.CCATC TGCTGCTGCC ...GGGAA.A TTCTTT..AA
Friedmannia           AGTGGGCGG. TTXX.CCGCT TGCTGCAGCC ...GAGAAXG TTCTCT..AA
Microthamnion         CTCGGGTGG. TTCC.ATCCG GCGCATTC.. ...GGGAA.G TTCGTT..AA
Myrmecia              .......... .......... .......... .......... ..........
Chlorosarcina         .......... .......... .......... .......... ..........
Pyramimonas.parkae    .......... .......... .......... .......... ..........
Pyramimonas.virgini   .......... .......... .......... .......... ..........
Mesostigma            .......... .......... .......... .......... ..........
Pedinomonas.tubercu   AGA....... .......... .......... .......... ..........
Pedinomonas.minor     ATCGGGGCAA GCTCGAACCT GTGTT..... ...GAGAA.T TTCGTT..GA
Micromonas            TTCTGAXXG. TTCG.CCGTC GGATGGCCTX ...GGGAA.G TTCGTT..XA
Mantoniella           .......... .......... .......... .......... ..........
Pseudoscourfieldia    .......... .......... .......... .......... ..........
Nephroselmis.pyrifo   GGTGGGTCC. GCCG.CXSTC CGCCCGT... ...CAGAA.G TTCTTC..AA
Pedinomonas.minutis   TCCTTCCTCG TGTTGGTTGT ACTTCGXX.. ...GRGAA.G TXATAC..AX
Costaria              .......... .......... .......... .......... ..........
Saccharomyces         GAAGGGGGCA ACTCCATCTC AAGCG..... ...GAGAA.T TTGGAC..AA
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Phaeodactylum       A.GTGCCTKT XTYGGTGTTG GTTGC..... ...GRGAA.C TTGTCT..AA

[                   1245            1264]
Glycine             ACCTTATC.A TTT.AGAGGA
Zamia               ACCTTATC.A TTT.AGAGGA
Equisetum           ACCTTACC.A TTT.AGAG..
Klebsormidium       ACCTTATC.A GTTTAGAGGA
Bryopsis            GCCAATTT.T CT..AXA...
Enteromorpha        ACCCTCXX.G TTT.AGAGXA
Chlamydomonas.eug   ACCCTCCC.A CCT.AGAG..
Chlamydomonas.moe   ACCCTCCC.A CCT.AGAG..
Chlamydomonas.rei   ACCCTCCC.A CCT.AGAGXA
Asteromonas         ACCCTCCCXA CCT.AGA...
Chlorella           ACCCUCCC.A CCU.AGAGGA
Nanochlorum         ACCcuCCC.A CCU.AgaGGa
Tetraselmis.carter  ACCCXCCC.C ATT.XXAG..
Tetraselmis.levis   ACC.XCCC.C ATTTXGAGAA
Pseudotrebouxia     ACCCTCCC.A CCT.XGAGXA
Pleurastrum         ACCCTCCC.A CCT.AGAGAA
Friedmannia         ACCCTCCC.A TCT.......
Microthamnion       ACCCTCCC.A CC........
Myrmecia            .......... ..........
Chlorosarcina       .......... ..........
Pyramimonas.parkae  .......... ..........
Pyramimonas.virgini .......... ..........
Mesostigma          .......... ..........
Pedinomonas.tubercu .......... ..........
Pedinomonas.minor   ACCCTCCC.A CCT.A.....
Micromonas          ACCTTATC.A ..........
Mantoniella         .......... ..........
Pseudoscourfieldia  .......... ..........
Nephroselmis.pyrifo ACCCTCGC.A TTT.AGAG..
Pedinomonas.minutis XCCTGATG.A TTW.AGAGGA
Costaria            .......... ..........
Saccharomyces       ACTTGGTC.A TTT.AGAGGA
Phaeodactylum       ACCTTATC.A TCT.AGAGGA

26C Primer

[                   1265                                              1314]
Glycine             ......GACC CTGA..TCTT CTG.TGAAGG GTTCGAGTGA GAGCATACCT
Zamia               CCGACCGACC CAGA..TCTT CTG.TGAAGG GCTCGAGTCC GAGCATACCT
Equisetum           TCGACCGACC GTGA..TCTT CTG.TGAAAG GTTTGAGTGA GAGCATACCT
Klebsormidium       .......... .......... .......... .......... ..........
Bryopsis            .......... .......... .......... .......... ..........
Enteromorpha        ...ACC.ACC .ATA..TCTT CTG.TGAAAG GTC.XAXTAC GASGTA.CCT
Chlamydomonas.eug   TC.ACXGACC .TAGA.GCTT CTG.CGAAAG GTTTGAGTXX TAGXXXXXAT
Chlamydomonas.moe   TCGACCGACC .TAGA.GCTT CTG.CGAAAG GTTTGAGTGC GAGCATAXAT
Chlamydomonas.rei   TCGACCGACX XTGTT.GTTT CTX.CGAAAG GTTTGAGT.C XAGCAXACCT
Asteromonas         .......... .......... .......... .......... GAGCATACCT
Chlorella           .......... .......... .......... .......... ..........
Nanochlorum         .......... .......... .......... .......... ..........
Tetraselmis.carter  TCGACCGACC .ATGA.TCTT TTG.TGAAAG GTTTGAGTAC GAGCATACCT
Tetraselmis.levis   .......ACC .ATGA.TCTT TTG.TGAAAG GTTTGAGTXC GAGCAKACCT
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Pseudotrebouxia     .......... .......... .......... .......... ..........
Pleurastrum         .......... .......CTT CTG.CGAAAG GTTTGAGTGC GAGCATACCT
Friedmannia         .......... .......... .......... .......... ..........
Microthamnion       .......... .ATGA.TCTT CTG.TGAAAG GTTTGAGTTC GAGCATACCT
Myrmecia            .......... .......... .......... .......... ..........
Chlorosarcina       .......... .......... .......... .......... ..........
Pyramimonas.parkae  .......... .......... .......... .......... ..........
Pyramimonas.virgini .......... .......... .......... .......... ..........
Mesostigma          .......... .......... .......... .......... ..........
Pedinomonas.tubercu .......... .......... .......... .......... ..........
Pedinomonas.minor   .......... ...GA.TCTT CTG.TGAAAG GTTTGAGTAC GAGCATACCT
Micromonas          .......... .......... .......... .......... ..........
Mantoniella         .......... .......... .......... .......... .........T
Pseudoscourfieldia  .......... ......TCTT CTG.TGAAAG GTTTGAGTRC GAGCRKACCT
Nephroselmis.pyrifo .......... ..TGA.TCTT CTG.TGAAAG GTTTGAGTRC GAGCAKACCT
Pedinomonas.minutis .......... .......... .......... .......... .AGCAGATAT
Costaria            .......... .......... .......... .......... ..........
Saccharomyces       .......... .......... .......... .......... ..........
Phaeodactylum       TCGACCGATC CTGATGTCTT CGGATGGA.. .TTTGAGTAA GAGCATAGCT

[                   1315                                                1364]
Glycine             GTCGGGACCC GAAAGATGGT GAACTRTGCC TGAGCGGGGC GAAGCCAGAG
Zamia               GTTGGGACCC GAAAGATGGT GAACTATGCC CGAGCAGGGT GAAGCCAGAG
Equisetum           GCTGGGACCC XAAAGATGGT GAACTATGCC TGAGCAGGGC GAAGCCAGAG
Klebsormidium       .......... ..XAGATGGT GAACTATGCC TGAGGCAGGC GAAGCCAGAG
Bryopsis            .......... .......... .......... .......... ..........
Enteromorpha        TTTGGGACCC TTAAGATGGT GAACTATGCC TGAKCA.GGC GAAGCCAGAG
Chlamydomonas.eug   XTTGGGACXX XXXXXATGGT GAACTATGCC TGGGCWGGGT GAAGCCAGAG
Chlamydomonas.moe   GTTGGGACCX XXXAGATGGT GAACTATGCC TGGGCAGGGT GAAGCCAGAG
Chlamydomonas.rei   GTTGGGACCX XX.AGATGGT GAACTATGCC TGAGCAGGGT GAAGCCAGAG
Asteromonas         GTTGGGACXX ...AGATGGT GAACTATGCC TGAGCAGGGT GAAGCCAGAG
Chlorella           .......... .......... .......... .......... ..........
Nanochlorum         .......... .......... .......... .......... ..........
Tetraselmis.carter  GTTGGGACCC XXXAGATGGT GAACTATGCC TGAGCA.GGC GAAGCCAGAG
Tetraselmis.levis   XTTGGGACCC XXXXGATGGT GAACTATGSC TGAGCA.GGC GAAGCCAGAG
Pseudotrebouxia     ...GGGXCCC XXXXGATGGT GXXCTATGSC TGAGCX.GGC SAAGCCAGAG
Pleurastrum         GTTGGGACCC GSAAGATGGT GAACTATGSC TGAGCA.GGC GAAGCCAGAG
Friedmannia         .......... ...AGATGGT GXXCTATGCC TGAGCX.GGC GAAGCSAGAG
Microthamnion       GTTGGGACCC XAAAGATGGT GAACTATGCC TGAGCA.GGC GAAGCCAGAG
Myrmecia            .......... .......... .......... .......... ..........
Chlorosarcina       .......... .......... .......... .......... ..........
Pyramimonas.parkae  .......... .......... .......... .......... ..........
Pyramimonas.virgini .......... .......... .......... .......... ..........
Mesostigma          .......... .......... .......... .......... ..........
Pedinomonas.tubercu .......... .......... .......... .......... ..........
Pedinomonas.minor   GTTGGGACCC XXXAGATGGT GAACTATGCC TGAGCA.GGC GAAGCCAGAG
Micromonas          ...GGGACCX XXXAGATGGT GAACTATGSS TGAGCA.GGC GAAGCCAGAG
Mantoniella         GCTGGGACCX XXX.GATGGT GAACTATGSC TGAGCA.GGC GAAGCCAGAG
Pseudoscourfieldia  GTTGGGACCC XXXAGATGGT GAACTATGSS TGAGCR.GGC GAAGCSAGAG
Nephroselmis.pyrifo GTTGGGACCX XXXXGATGGT GAACTATGCC TGAGCA.GGC GAAGCCAGAG
Pedinomonas.minutis GTTGGKXCCC XXXAGATGGT GAACTATGSC TGAACXGGGT GAAGCCAGGG
Costaria            .......... .......... .......... .......... ..........
Saccharomyces       .......... .......... .......... .......... ..........
Phaeodactylum       GTTGGGACCC GAAAGATGGT GAACTATGCC TGAATAGGGT GAAGCCAGAG
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[                    1365                                                1414]
Glycine              GAAACTCTGG TGGAGGCCCG CAGCGATACT GACGTGCAAA TCGT.TCGTC
Zamia                GAAACTCTGG TGGAGGCCCG TAGCGATACT GACGTGCAAA TCGT.TCGTC
Equisetum            GAAACTCTGG TGGAGGCTCG TAGCGATACT GACGTGCAAA TCGT.TCGTC
Klebsormidium        GAAACTCTGG TGGAGGCTCG XAXCGATACT GACGTGCAAA TCGT.TCGTC
Bryopsis             .......... .......... .......... .......... ..........
Enteromorpha         GAAACTCTGG TGKAGGCTCG TAGATGTGCT .ACGTGCAAA TCGCCTTTTC
Chlamydomonas.eug    GAAACTCTGG TGGAGGCTCG .XGATGTGCT .ACGTGCAAA TCGC.TTTTC
Chlamydomonas.moe    GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCAAA TCGC.TTTTC
Chlamydomonas.rei    GAAACTCTGG TGGAGGCTCG TAGATGTGCT .AXGTGCAAA TXXC.TTTTC
Asteromonas          GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCAAA TCGC.TTTTC
Chlorella            .......... .......... .......... .......... ..........
Nanochlorum          .......... .......... .......... .......... ..........
Tetraselmis.carter   GAAACTCTGG TGGAGGCTCG TAGATGTGCT XACGTGCAAA TCGC.TTTTC
Tetraselmis.levis    GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCAAA TCGC.TTTTC
Pseudotrebouxia      GAAACTCTGG TGSAGGCTCG KAGATGTGCT GACGTGCXRA TCGC.TTTTC
Pleurastrum          GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCSAA TCGC.TTTTC
Friedmannia          GAXXXTCTGG TGGAGGCTCG KAGATGTGCT GACGTGCXAA TCGC.TTTTC
Microthamnion        GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCAAA TCGC.TTTTC
Myrmecia             .......... .......... .......... .......... ..........
Chlorosarcina        .......... .......... .......... .......... ..........
Pyramimonas.parkae   .......... .......... .......... .......... ..........
Pyramimonas.virgini  .......... .......... .......... .......... ..........
Mesostigma           .......... .......... .......... .......... ..........
Pedinomonas.tubercu  .......... .......... .......... .......... ..........
Pedinomonas.minor    GAAACTCTGG TGGAGGCTCG TAGATGTGCT GACGTGCAAA TCGC.TTTTX
Micromonas           GAAACTCTGG TGGAGGCTCG TAGCGATACT GACGTGCXAA TCGT.TCGTC
Mantoniella          GAAACTCTGG TGGAXXCTCG TXGCGATACT GACGTGCXAA TCGT.TCGTC
Pseudoscourfieldia   GAAACTCTGG TGGAGGCTCG TAGCGATACT GACGTGCXAA TCGT.TCGTS
Nephroselmis.pyrifo  GAAACTCTGG TGGAXXCTCG TAXXGRTACT GACGTGCAAA TCGT.TCGTC
Pedinomonas.minutis  GAAACTSTGG TGGAGGCTCG TAGCKATACT GACGAGCXAA TCGT.TCGTS
Costaria             .......... .......... .......... .......... ..........
Saccharomyces        ....CTCTGG TGGAGGCTCG TAGCGATTCT GACGTGCXXA TCGA.TCGTC
Phaeodactylum        GAAACTCTGG TGGAGGCTCG TAGCGGTTCT GACGTGCAAA TCGA.TCGTC

[                    1415                                                1464]
Glycine              TGA.CTT.GG GTATAGGGGC G.AAAGACTA ATCGAACCGT CTAGTAGCTG
Zamia                TGA.CTT.GG GTATAGGGGC G.AAAGACTA ATCGAACCGT CTAGTAGCT.
Equisetum            AGA.CTT.GG GTATAGGGGC G.AAAGACTA ATCGAACCAT CTA.......
Klebsormidium        AGX.CTT.GG GKRTAXXXGC GGAAAGACTA ATCGAACCAT CTAGXTA...
Bryopsis             .......... .......... .......... .......... ..........
Enteromorpha         GGA.CTT.GG GTATAGG.XC X.AAAGACTA ATCGAACCAT CTAGTAGCXG
Chlamydomonas.eug    TGA.CCT.GG GCATAGGGGC G.AAAGACTT ATXKAACCAT CTAGTAGCTG
Chlamydomonas.moe    TGA.CCT.GG GCATAGGGGC G.AAAGACTA ATCGAACCAT CTAGTA....
Chlamydomonas.rei    AGA.CTT.GG GTATAGGGGC G.AAAXACTA ATCGAACCAT CTAGTAGCT.
Asteromonas          GGA.CTT.GG GXATAGGGGC G.AAAGA... .......... ..........
Chlorella            .......... .......... .......... .......... ..........
Nanochlorum          .......... .......... .......... .......... ..........
Tetraselmis.carter   GGA.CTT.GG GKRTAXXXXC G.AAAGACTX ATCXAACCAT CTA.......
Tetraselmis.levis    GGA.CTT.GG GKATAGGGGC X.AAAGACTA ATCGAACCAT CTA.......
Pseudotrebouxia      GGA.CTT.GG GXXTAXXGGC G.XXAGACTA ATCGAACCAT CTAGTAGCT.
Pleurastrum          GGA.CTT.GG GXXTAXGGGC G.RRRGACTA ATCGAACCAT CTAGTAXCT.
Friedmannia          SGA.CTT.GX GKMTAGGGGC G.AAAGACTC ATCSAACCAT CTAGTAXC..
Microthamnion        XGA.CTT.GG GTATAGGGGC G.AAAGACTA ATCGAACCAT CTAG......
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Myrmecia                .......... .......... .......... .......... ..........
Chlorosarcina           .......... .......... .......... .......... ..........
Pyramimonas.parkae      .......... .......... .......... .......... ..........
Pyramimonas.virgini     .......... .......... .......... .......... ..........
Mesostigma              .......... .......... .......... .......... ..........
Pedinomonas.tubercu     .......... .......... .......... .......... ..........
Pedinomonas.minor       GGA.CTT.GG GTATAGGGGC G.AAAGACTA ATCGAACTAT CTA.......
Micromonas              GGA.CTT.GG GTATAXXXXX X.XAAGACTX ATCXAA.... ..........
Mantoniella             GGA.CTT.GG GTATAXXXXC X.AAAGACTX ATCXAACCAT CTAXTAXCT.
Pseudoscourfieldia      GGA.CTT.GX GXXXAXXXXC G.RRAGACTR ATCGAACCAT CTAGTA....
Nephroselmis.pyrifo     GGA.CTT.GG GTATAXGGXC G.AAAGACTX ATCXAACCAT CTAXTA....
Pedinomonas.minutis     TGA.TTT.GG GKATAGGGGC G.XAAGACTA ATCGAACCAT CTAGTA....
Costaria                .......... .......... .......... .......... ..........
Saccharomyces           GAAATTTGXG GTATAGGGGC G.AAAGACTG ATCGAACTAT CTAGTAXCTG
Phaeodactylum           GAA.TTT.GG GTATAGGGGC G.AAAGACTA ATCGAACCAT CTAGTAGCTG

26D Primer
[                       1465                                           1514]
Glycine                 GGGAGTCCGG AGACGTCGGC GGGGGCCCCG GAAAGAGTTA TCTTTTCTGT
Zamia                   GGGAATCCGG AGACGTCGGC GGGGGCCCCG TGAAGAGTTA TCTTTTCCTT
Equisetum               CTGAATCCAG AGACGCCGGC GGGGGCCCTG GGAAGAGTTC TCTTTTCTTT
Klebsormidium           .......... .......... .......... .......... ..........
Bryopsis                TCAACCCCGG AGATGCCGGC GGCGGTGGCG GGAAGAGTTC TCTTTTCTTS
Enteromorpha            .......... .......... ...SGGWYTG GAAAGAGTTC TCTTT.CTT.
Chlamydomonas.eug       .......... ......TCGG TATGGCCCTG GGAAGAGTTC TCTTTTCTTT
Chlamydomonas.moe       .......... .......... .......... .......... ..........
Chlamydomonas.rei       .......... .......... .......... .......... ..........
Asteromonas             .......... .......... .......... .......... ..........
Chlorella               .......... .......... .......... .......... ..........
Nanochlorum             .......... .......... .......... .......... ..........
Tetraselmis.carter      .CGAGCTCAC AGACGTC.GC GATGGCCCTG GGAAGAGTTC TCTTTTCTTT
Tetraselmis.levis       .CGAGCTCAC AGACGTCGGC GATGGCCCTG GGAAGAGTTC TCTTTTCTTT
Pseudotrebouxia         .......... .......... .......... .GGAGAGTTC TCTTTTCTKT
Pleurastrum             .......... .......... ...GGCCSTG GGARGAGTTC TCTTTTCTKT
Friedmannia             .......... .......... .......... .GGAGAGTTC TCTTTTCTTC
Microthamnion           .......... .......... ATCGGCCCTG GGAAGAGTTC TCTTTTCTTT
Myrmecia                .......... .......... .......... .......... ..........
Chlorosarcina           .......... .......... .......... .......... ..........
Pyramimonas.parkae      .......... .......... .......... .......... ..........
Pyramimonas.virgini     .......... .......... .......... .......... ..........
Mesostigma              .......... .......... .......... .......... ..........
Pedinomonas.tubercu     .......... .......... .......... .......... ..........
Pedinomonas.minor       .......... .......... .....CCCTG GGAAGAGTTC TCTTTTCTTT
Micromonas              .......... .......... .GGGAGCCCC GGGAGAGTTC TCTTTTCTTT
Mantoniella             .......... .......... .......... ...AGAGTTC TCTTTTCTTT
Pseudoscourfieldia      .......... .......... .GGGGCCCCA GGAAGAGTTC TCTKTTCTKT
Nephroselmis.pyrifo     .......... .......GGC GGGGGCCCCA GGAAGAGTTC TCTTTTCTTT
Pedinomonas.minutis     .......... .......... .......TCC GGXXGAGTTX TCTTTTCTTT
Costaria                .......... .......... .......... .......... ..........
Saccharomyces           .......... .......... ..GGGCCCGG ACGAGAGTTT TCTTTTCTTG
Phaeodactylum           CTGAATGTGG AGACGTCGGC GCGAGCCCTG GGAGGAGTTA TCTTTTCTTC

[                       1515                                           1564]
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Glycine                TTAA.CA.GC C.TGCCCACC CTGGAAA..G CCTCAGCCGG AGGTAGGGTC
Zamia                  TTAA.CA.GC C.TGCCCACC CTGGAATC.G GTTCAATCGG AGATAGGGTC
Equisetum              TTAA.CA.AC T.TGCCCACC CTGAAATC.G GATCAACCGG AGATAGGGTC
Klebsormidium          .......... .......... .......... .......... ..........
Bryopsis               TTGA.CG.GT C.CSAGTGCC TTGGAATC.C ATTCGATGGG AGATAAGGCT
Enteromorpha           TTAA.CA.GC C.CCAYGACC CTGGAATC.G AGTCATTCGG AGATAGGGTT
Chlamydomonas.eug      TTRA.CA.GC T.CGAAAGCC CTGGAATC.G AATCXTTCGG AGATAGGGCC
Chlamydomonas.moe      ..CA.CC.GC T.CGAAAGCC CTGGAATC.G XATXATTCGG AGATCGGGCT
Chlamydomonas.rei      .......... .......... .......... .......... ..........
Asteromonas            .......... .......... .......... .......... ..........
Chlorella              .......... .......... .......... .......... ..........
Nanochlorum            .......... .......... .......... .......... ..........
Tetraselmis.carter     TTAA.CAGGC T.CGAAGGCC CTGGAATC.T AATCATTAGG AGATAGGGCT
Tetraselmis.levis      TTAA.CGAGC T.CGAAGGCC CTGGAATC.T AATCXTTAGG AGATAGGGCT
Pseudotrebouxia        TTMA.CA.GC T.SGAAGGCC CTGGAATC.G GCTSATCCGG AGAKAGGGCC
Pleurastrum            TTRA.CA.GC K.CGAAGGCC CTGGAATC.G AATCRKTCGG AGAKAGGGCT
Friedmannia            TTAA.CA.AC C.CGAAGGSC CTGGAATC.G GCTCATCCGG AGATAGGGCT
Microthamnion          TTAA.CA.GT T.CGAAGGCC CTGGAATC.G GATCATCCGG AGATAGGGCT
Myrmecia               .......... .......... .......... .......... ..........
Chlorosarcina          .......... .......... .......... .......... ..........
Pyramimonas.parkae     .......... .......... .......... .......... ..........
Pyramimonas.virgini    .......... .......... .......... .......... ..........
Mesostigma             .......... .......... .......... .......... ..........
Pedinomonas.tubercu    .......... .......... .......... .......... ..........
Pedinomonas.minor      TTAA.CXXXC T.CGAAAGCC CTTGAATC.G GATCXTCCGG AGATGGGGCT
Micromonas             TTXAXCA.GC C.KTCGCGCC CTGGAATC.G GTTTRGCCGG AGATA.GGCC
Mantoniella            TTXA.CA.GC C.TTSGCGCC CTGGAATC.G GTTTXGCCGG AGATA.GGCC
Pseudoscourfieldia     TTRA.CR.GC C.SGCCCRSC CTGGAATSXG GATTRTCCGG AGAKA.GGSC
Nephroselmis.pyrifo    TTAA.CA.GC C.CGCCCACC CTGGAATC.G GATTATCCGG AGATA.GGCC
Pedinomonas.minutis    TTXA.CA.GC C..TGTAGCC CTGGAATC.G GATTXCCCGG AKATAGGGTG
Costaria               .......... .......... .......... .......... ..........
Saccharomyces          TTAA.CAAGC GASRCCGACC CCGGAATC.G GATTGCCCGG AGATGGGGTT
Phaeodactylum          TTAA.CA.GC T..TATCACC CCGGAATT.G GTTTATCCGG AGATGGGGTC

[                      1565                                              1614]
Glycine                CAGCGGCTGG A.AGAGCACC GCACGTCGCG T.GGT.GTCC GGT....GCC
Zamia                  CAGCGGCTGG A.AGAGCACC GCACGTCCCG T.GGT.GTCC GGT....GCC
Equisetum              CAGCGGTTGG T.AAAGCAC. GCAGGTCXTG C.GGT.GTCC GGT....GCC
Klebsormidium          .......... .......... .......... .......... ..........
Bryopsis               TGGCGATCGG T.ATAGCGCC GCCAGTTGTT GCGTGTGTCC GCCTCGCCTC
Enteromorpha           CAGTGCCTGG TAXAAGCACA MSTC.TST.. ..GGT.GTCC GGCGCGCAGT
Chlamydomonas.eug      TAGCAGCTGG A..AAGCATC TCA.XTTTA. ..GGT.GTC. ..........
Chlamydomonas.moe      TXGCCGCTGG G.AAAGCCTC TXA.GTTTTG A.GGT.GTCC CGTGC..GCC
Chlamydomonas.rei      .......... .......... .......... .......... ..........
Asteromonas            .......... .......... .......... .......... ..........
Chlorella              .......... .......... .......... .......... ..........
Nanochlorum            .......... .......... .......... .......... ..........
Tetraselmis.carter     CAGAAGTCGG T.AAAGCACC GCACGTCKCG C.GGK.GTCC GGAG..CGCC
Tetraselmis.levis      CAGAAGTCGG T.AAAGCACC GCACGTCTCG C.GGT.GTCC GGAG..CGCC
Pseudotrebouxia        CAKAAGCTGG T.MAAGCACT GCACTTCTCG GCAGT.GTCC GGA...GCCC
Pleurastrum            CAGAAGCTGG T.AAAG.MC. GCASGTCTSG C.GGTXGTCS GCG...CGCS
Friedmannia            CRGAGGTTXG T.AAAGCACT GCACTXXXXG C.AGT.XTCC GGAGX.CGCC
Microthamnion          CAGCAGCTGG T.AAAGCACC GCACGTCTCG C.GGT.GTCC GGCG..CGCC
Myrmecia               .......... .......... .......... .......... ..........
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Chlorosarcina          .......... .......... .......... .......... ..........
Pyramimonas.parkae     .......... .......... .......... .......... ..........
Pyramimonas.virgini    .......... .......... .......... .......... ..........
Mesostigma             .......... .......... .......... .......... ..........
Pedinomonas.tubercu    .......... .......... .......... .......... ..........
Pedinomonas.minor      CAGCAGCGGG G.AAAGCACT GCATCTTTCG C.AGT.GTCC GGTG..CG..
Micromonas             CAACGGCTGG T.AAAGCACT GCACGTSTCG C.AGT.GTCC GGTG..CXCT
Mantoniella            CAACGGCTGG T.AAAGCACT GCAXGTXXCG C.AGT.GTCC GGTG..CACT
Pseudoscourfieldia     CRGCGGCTGG K.RAAG.MC. GCACTTCTTG C.GGR.GTCT GGTG..XXCC
Nephroselmis.pyrifo    CAGCGGCTGG T.AAAG.MC. .CACTXCTTG C.GGX.GTCT GGTG..CGCC
Pedinomonas.minutis    ATGTGGCTGG T.AAAGCACC TCACGTCTTG A.GGT.GTXC KGTG..CSTC
Costaria               .......... .......... .......... .......... ..........
Saccharomyces          GGCGGCTTXG T.AAASGGCC GCTXXTTGGC SG....CTTC GGTG..CGCG
Phaeodactylum          TTATGGCTGG A.AGAGGCCA GCACCTTTGC TGG...CTCC GGTG..CGCT

[                      1615                                            1664]
Glycine                CCCGG.CGGC CCTTGAAAAT CCGGAGGACC GAGTGCCTCC CACGCCCGGT
Zamia                  CCCGG.CGGC CCTTGAAAAT CTGGAGGACC GAGTACCGTC C.........
Equisetum              CCCGG.CGGC CCTTGAAAAT CTGGAGGAGC G.ATTACTXA TC........
Klebsormidium          .......... .......... .......... .......... ..........
Bryopsis               CGACGGCCCK TGAXAAAXTC SGGGGGAGTG AAXTCXC... ..........
Enteromorpha           CGAGT.CGGT CCGTGAAAAT GTAGGXXA.G C.ATTCGCGA TC.TTGCC..
Chlamydomonas.eug      .......... .......... .......... .......... ..........
Chlamydomonas.moe      GATMA.CGAT CCTTXXXXAT CCGGGXGAGT GAATMC.CGA TC.TGTMA..
Chlamydomonas.rei      .......... .......... .......... .......... ..........
Asteromonas            .......... .......... .......... .......... ..........
Chlorella              .......... .......... .......... .......... ..........
Nanochlorum            .......... .......... .......... .......... ..........
Tetraselmis.carter     ATTGA.CGAT CCTTGAAAAT XTGAGGGASS XXATXTTACA CTTCTT....
Tetraselmis.levis      ATTGA.CGAT CCKTGAAAAG TGTAGGGASS SS.TSTTACA CTTCTTXCCA
Pseudotrebouxia        ACCGA.CGGC CCKTGARAAK CXXXXGGM.C CAAXTMCTXA KCTKTGCCA.
Pleurastrum            GATXA.CGGT CCTKGARAAT .......... .......... ..........
Friedmannia            ATTGAGCGGC CCTTGAAAXK TTGGGGGAXX XXXATXXCTG TTCTTGCCA.
Microthamnion          GATGA.CGGT CCTTGAAAAT CCGAGGGA.. GCGGCTACXG ATCTTGC...
Myrmecia               .......... .......... .......... .......... ..........
Chlorosarcina          .......... .......... .......... .......... ..........
Pyramimonas.parkae     .......... .......... .......... .......... ..........
Pyramimonas.virgini    .......... .......... .......... .......... ..........
Mesostigma             .......... .......... .......... .......... ..........
Pedinomonas.tubercu    .......... .......... .......... .......... ..........
Pedinomonas.minor      .......... .......... .......... .......... ..........
Micromonas             CCXGG.XGGC CCXTGAAAA. .......... .......... ..........
Mantoniella            CCCGA.CGGC CCXXXAAAAX CTA....... .......... ..........
Pseudoscourfieldia     CTCGA.CGGC CCGTGAAAAT CTGGGGGA.. XXGAKKACCG TT........
Nephroselmis.pyrifo    CTCGA.CGGC CCXXGXAAAT C......... .......... ..........
Pedinomonas.minutis    TCXXA.CXCX XCTTGAAAAG GCXXXXXAAX GATTXTTT.. ..........
Costaria               .......... .......... .......... .......... ..........
Saccharomyces          CTSGA.CGCT CCTTGAAAAC CTGGCASSGT GTTTTATTCX CACCCCT...
Phaeodactylum          TGTGA.CGGC CCGTGAAAAT CCACAGGAAG GAATAGTTTT CATGCTAGGT
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APPENDIX B

ALIGNED ITS1, 5.8S, AND ITS2 SEQUENCES

Appendix B. Aligned sequences of the ITS1, 5.8S rDNA, and ITS2 regions included in the phylogenetic analysis. Abreviations for the taxa are as follows: Tchuii = Tetraselmis chuii, UW421 = Tetraselmis tetrahele, UW494 = Tetraselmis tetrahele, Platy1 = Tetraselmis levis, UW490 = Tetraselmis striata, 2286 = Tetraselmis suecica, UW480rc = Tetraselmis verrucosa, CC1952 = Chlamydomonas reinhardtii, Vfarrs58s = Vicia faba (Nazar and Wildeman 1981), Vfarrb = Vicia faba (Tanaka et al. 1980), Whttrb = Triticum vulgare (MacKay et al. 1980), Whtrrb1 = Triticum vulgare (Wildeman and Nazar 1982), Yscrrb = Saccharomyces cerevisiae (Rubin 1973).

```
            1                                                  50
    Tchuii  .......... .......... .....TTGAG CGTAGGGATG CGTTCCCTAG
      Uw421 .......... .......... ......TGAG CGTAGGGATG CGTTCCCTAG
      Uw494 .......... .......... .....CTCAC CGCCCTCAAA CACGGGGCGC
      Platy1 .......... .......... .......... ....CTCAAA CACGGGGCXC
      Uw490 GCGGCTGGGA TGCGTTCCCA GTCGGCTCAC CGCCCTCAAA CACGGGGCGC
       2286 .......... .......... ..CGGCTCAC CGCCCTCAAA CACGGGGCGC
    Uw480rc .......... .......... .......... .......... ..........
      Cc1952 .......... .......... .....CCAGG TCTGGGCGCA ATGTAAAAGT
  Vfarrs58s .......... .......... .......... .......... ..........
      Vfarrb .......... .......... .......... .......... ..........
      Whtrrb .......... .......... .......... .......... ..........
     Whtrrb1 .......... .......... .......... .......... ..........
      Yscrrb .......... .......... .......... .......... ..........

            51                                                 100
    Tchuii  TCGGGCCTAC CCCCGCGCCT CAG..XAATA GGTCGGCGCT CT.TAAACAA
      Uw421 TCGGGCCTAC CCCCGCGCCT CAGCGATATA GGTCGGCGCT CT.TAAACAA
      Uw494 TCCTATTAAC TTAGGCGTCT CGGGCGGCTG GGCTGGCGTT ATTTAAACAC
      Platy1 TCCTATTAAC TTAGGCGTCT CGGGCGGCTG GGCTGGCGTT ATATAAACAC
      Uw490 TCCTATTAAC TTAGGCGTCT CGGGCGGCTG GGCTGGCGTT ATTTAAACAC
       2286 TCCTATTAAC TTAGGCGTCT CGGGCGGCTG GGCTGGCGTT ATTTAAACAC
    Uw480rc ....CTAGGC TTTTXXXXXA CCAGCTGGCT AXXCGCCAAA CCAAATTCAC
      Cc1952 TACGCCTGGC CTGGGTTGCC GCACAGTCGG TCTCTTATAC TAACCAACCA
  Vfarrs58s .......... .......... .......... .......... ..........
      Vfarrb .......... .......... .......... .......... ..........
      Whtrrb .......... .......... .......... .......... ..........
     Whtrrb1 .......... .......... .......... .......... ..........
      Yscrrb .......... .......... .......... .......... ..........

            101                                                150
    Tchuii  CCCACACAAA AACAACGTCT AAAGCTATGT GTATG.TTGG CCTTGCGACT
      Uw421 CCCACACAAA AACAACGTCT AAAGCTATGT GTATG.TTGG CCTTGCGACT
      Uw494 TCCACACCAA AACAACGTCT AAAGCTATGT GCGTA.TTGC CCCGTGCGAT
      Platy1 TCCACACCAA AACAACGTCT AAAGCTATGT GCGTA.TTGC CCCGTGCGAT
      Uw490 TCCACACCAA AACAACGTCT AAAGCTATGT GCGTA.TTGC CCCGTGCGAT
       2286 TCCACACCAA AACAACGTCT AAAGCTATGT GCGTA.TTGC CCCGTGCGAT
    Uw480rc TCAACACAAA AACAA.GTCT GAAGCTATTT .CTGATTGAC CCAGT.CGAT
      Cc1952 ACACCAAACC AAAACTAAAT TYAAACCGAG TATCTAGCTT AGAGCTAGTG
  Vfarrs58s .......... .......... .......... .......... ..........
      Vfarrb .......... .......... .......... .......... ..........
      Whtrrb .......... .......... .......... .......... ..........
     Whtrrb1 .......... .......... .......... .......... ..........
      Yscrrb .......... .......... .......... .......... ..........

            151                                                200
    Tchuii  GCATC.TAAC C..AAAGAC. AACTCTCAAC AACGGATATC TT.GGCTCTT
      Uw421 GCATC.TAAC C..AAAGAC. AACTCTCAAC AACGGATATC TTCGGCTCTT
      Uw494 ACCGCCTAAC C..AAAGAC. .ACTCTCAAC AACGGATATC TT.GGCTCTT
      Platy1 ACCGCCTAAC C..AAAGAC. AACTCTCAAC AACGGATATC TT.GGCTCTT
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          Uw490  ACCGCCTAAC C..AAAGAC. AACTCTCAAC AACGGATATC TT.GGCTCTT
           2286  ACCGCCTAAC C..AAAGACX AACTCTCAAC AACGGATATC TT.GGCTCTT
         Uw480rc CAGTC.TAAC CA.TAAGAC. AACTCTCAAC AACGGATATC TT.GGCTCCT
          Cc1952 CTCAC.TAAC CA...AGXX. AACTCTCAAC AACGGATATC TT.GGCTCTC
       Vfarrs58s .......... ....AGAAT. GACTCTCGGC AACGGATATC TA.GGCTCTT
          Vfarrb .......... ....AGAAT. GACTCTCGGC AACGGATATC TA.GGCTCTT
          Whtrrb .......... ....CACAC. GACTCTCGGC AACGGATATC TC.GGCTCTC
         Whtrrbl .......... ....CACAC. GACTCTCGGC AACGGATATC TC.GGCTCTC
          Yscrrb .......... ........A. AACTTTCAAC AACGGATCTC TT.GGTTCTC

                 201                                                 250
          Tchuii ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
           Uw421 ACAACCATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
           Uw494 ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
          Platy1 ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
           Uw490 ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
            2286 ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
         Uw480rc ACAACGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTCGAGAA
          Cc1952 GGATCGATGA AGAACGCAGC GAAATGCGAT ACGTAGTGTG AATTGCAGAA
       Vfarrs58s GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA
          Vfarrb GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA
          Whtrrb GCATCGATGA AGAACGTAGC GAAATGCGAT ACCTGGTGTG AATTGCAGAA
         Whtrrbl GCATCGATGA AGAACGTAGC GAAATGCGAT ACCTGGTGTG AATTGCAGAA
          Yscrrb GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATGTG AATTGCAGAA

                 251                                                 300
          Tchuii TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AG...CCTCG
           Uw421 .......... .......... .......... .......... ..........
           Uw494 TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AG...CCTCG
          Platy1 TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AGG..CCTCG
           Uw490 TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AG...CCTCG
            2286 TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AG...CCTCG
         Uw480rc TTCCGTGAAC CATCGAATCT TTGAACGCAT ATTGCGCTCG AGG..CCTCG
          Cc1952 ATAGCTCAAT CATCGAATCT TTGAACGCAT ATTGCGCTCG AGG..CTTCG
       Vfarrs58s TCCCGTGAAC CATCGAGTCT TTGAACGCAA GTTGCGCCCG ATGCCATTAG
          Vfarrb TCCCGTGAAC CATCGAGTCT TTGAACGCAA GTTGC.CCCG ATGCCATTAG
          Whtrrb TCCCGCGAAC CATCGAGTCT TTGAACGCAA GTTGCGCCCG AG.CCACTCG
         Whtrrbl TCCCGCGAAC CATCGAGTCT TTGAACGCAA GTTGCGCCCG AGGCCACTCG
          Yscrrb TTCCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCC. .....CTTGG

                 301                                                 350
          Tchuii GCCAAGAGCA CGCCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
           Uw421 ...XXXXXXX XXXCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
           Uw494 GCCAAGAGCA CGCCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
          Platy1 GC.AAGAGCA CGCCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
           Uw490 GCCAAGAGCA CGCCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
            2286 GCCAAGAGCA CGCCTGCCTC ....AGAGTC GGTTTTCCCC CTCAACCCCC
         Uw480rc GCCAAGAGCA CGCCTGCCTC ....AGGGTC ATGATTACC. .TCACCCC..
          Cc1952 GC.AAGAGCA TGTCTGCCTC ....AGCGTC GGGTTAATAC .TCGCCC...
       Vfarrs58s GTTGAGGGCA CGTCTGCCTG ....GGTGTC ACAT...... ..........
          Vfarrb GTTGAGGGCA CGTCTGCCTG ....GGTGTC ACAT...... ..........
          Whtrrb .CCGAGGGCA CGCCTGCCTG ....GGCGTC ACGC...... ..........
         Whtrrbl GCCGAGGGCA CGCCTGCCTG ....GGCGTC ACGC...... ..........
          Yscrrb TATTCCAGGG GGCATGCCTG TTTGAGCGTC ATTT...... ..........
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            351                                                        400
   Tchuii   C..TACCCC. ..........  .......TTA GG..TAGAGC CGGTT.GGAC
    Uw421   C..TACCCC. ..........  .......TTG GG..TAGAGC CGXTT.GGAC
    Uw494   C..TACCC.. ..........  .......TTG GG..TAGAGC CGGTT.GGAC
   Platyl   C..AACCCCC C.......TT CACCGGGGG. CG..GGCGGC GGATT.GGAC
    Uw490   C..AACCCCC ........TT CACCGGGGG. CG..GGCGGC GGATT.GGAC
     2286   CACAACGCCC C.......TT CACCGGGGGG CG..GGCGGC GGATT.GGAC
  Uw480rc   ...TACCTAC C.........  ........TA GG..TAT... CGGGT.GGAC
   Cc1952   ...TACTCCA ACAC...XXX XXXXXXXTTG TGTTTGGAGC AAGAGCGGAC
 Vfarrs58s  .......... ..........  .......... .......... ..........
    Vfarrb  .......... ..........  .......... .......... ..........
    Whtrrb  .......... ..........  .......... .......... ..........
   Whtrrbl  .......... ..........  .......... .......... ..........
    Yscrrb  .......... ..........  .......... .......... ..........

            401                                                        450
   Tchuii   CTGGCAGTCT CAGAGCTTTC ATT.......  .AGCGCTGGG TCTGCTGAAG
    Uw421   CTGGCAGTCT CAGAGCTTTC ACC.......  .AGCGCTGGG TCTGCTGAAG
    Uw494   CTGGCAGTCT CAGAGCTTTC ATT.......  .AGCGCTGGG TCTGCTGAAG
   Platyl   CTGGCAGTCT CATTGGCAGC AAT.......  .GCGTATGGG TCTGCTGAAG
    Uw490   CTGGCAGTCT CATTGGCAGC AAT.......  .GCGCATGGG TCTGCTGAAG
     2286   CTGGCAGTCT CATTGGCAGC AAT.......  .GCGCATGGG TCTGCTGAAG
  Uw480rc   CTGGCCTCCT CACCCGCAAG GGT.......  .......GGG CTGGCTGAAG
   Cc1952   CTGGCTGTCT CGGTGTTTGA TTTTCGGATC AGACGCCGGG TCAGCTGAAG
 Vfarrs58s  .......... ..........  .......... .......... ..........
    Vfarrb  .......... ..........  .......... .......... ..........
    Whtrrb  .......... ..........  .......... .......... ..........
   Whtrrbl  .......... ..........  .......... .......... ..........
    Yscrrb  .......... ..........  .......... .......... ..........

            451                                                        500
   Tchuii   TGCAGAGATT TAACCG.GGA CCC.GCTAAG GG........  ..........
    Uw421   TGCAGAGATT TAACCG.GGA CCC.GCTAAG GGXAAACACT AGGTAGGTAG
    Uw494   TGCAGAGATT TAACCG.GGA CCC.GCTAAG GG........  ..........
   Platyl   TGCAGAGATC CAGACA.GGA CCCTATTATG GG........  ..........
    Uw490   TGCAGAGATC CAGACA.GGA CCCTATTATG GG........  ..........
     2286   TGCAGAGATC CAGACA.GGA CCCTATTATG GGXAAACACT AGGTAGGTAG
  Uw480rc   TGCAGAGATC GAACCA.CTG CCATATCTTG GXXXCCCCTG GAGTGCCTCG
   Cc1952   TACAGAGGTT GATGCATGGA CCCGCTTATG GGXCTCTACT GGGTAGGCAA
 Vfarrs58s  .......... ..........  .......... .......... ..........
    Vfarrb  .......... ..........  .......... .......... ..........
    Whtrrb  .......... ..........  .......... .......... ..........
   Whtrrbl  .......... ..........  .......... .......... ..........
    Yscrrb  .......... ..........  .......... .......... ..........

            501                                                        550
   Tchuii   .......... ..........  .......... .......... ..........
    Uw421   GC........ ..........  .......... .......... ..........
    Uw494   .......... ..........  .......... .......... ..........
   Platyl   .......... ..........  .......... .......... ..........
    Uw490   .......... ..........  .......... .......... ..........
     2286   GCTT...... ..........  .......... .......... ..........
  Uw480rc   GCACCCAGGT GGTCTTGGGG GCGAGCTCCG GTAGGTAGCC TAAGGGTTAT
   Cc1952   CTCGTTGCTX ATGCTTTAGT AGATGGCTTG XAGCTGTGCT TGTCGACCCA
```

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Vfarrs58s  .......... .......... .......... .......... ..........
   Vfarrb  .......... .......... .......... .......... ..........
   Whtrrb  .......... .......... .......... .......... ..........
  Whtrrb1  .......... .......... .......... .......... ..........
   Yscrrb  .......... .......... .......... .......... ..........

           551                                      577
   Tchuii  .......... .......... .......
    Uw421  .......... .......... .......
    Uw494  .......... .......... .......
   Platy1  .......... .......... .......
    Uw490  .......... .......... .......
     2286  .......... .......... .......
  Uw480rc  T......... .......... .......
    Cc1952 AACCAGXAAC TTTGGCCCTG TGCCGAA
Vfarrs58s  .......... .......... .......
   Vfarrb  .......... .......... .......
   Whtrrb  .......... .......... .......
  Whtrrb1  .......... .......... .......
   Yscrrb  .......... .......... .......
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VITA

Thomas Sinclair Kantz was born in Austin, Texas on October 1, 1962 to Prudence Ann and Paul Thomas Kantz, and he grew up in Sacramento, California. Tom earned his B.S. in Botany at the University of California, Berkeley in 1984, and his M.S. in Botany at the University of Texas, Austin in 1987. He began his Ph.D. work in 1987, and his interests include algal systematics, the biology of the Prasinophyceae and the Pleurastrophyceae (Chlorophyta), and molecular evolution. In the Fall of 1992, Tom started collaboration with Dr. Marvin Fawley of the Department of Botany, North Dakota State University, Fargo, North Dakota, working on the development of fluorescent labelled oligonucleotide probes, "phylogenetic stains," for identification of coccoid prasinophyte algae from field collections. Tom is currently an Instructor of Biology in the Department of Biology, University of South Carolina, Coastal Carolina College, Conway, South Carolina. He is married to Dr. Katherine Lynn Taylor.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Thomas Sinclair Kantz

Major Field: Botany

Title of Dissertation: The Phylogeny of the Prasinophyceae and Pleurastrophyceae (Chlorophyta) Inferred from Ribosomal RNA Genes and Morphology

Approved:

Russell L Chapman
Major Professor and Chairman

Daniel Fogel
Dean of the Graduate School

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April 9, 1992